JOURNAL OF AGRICULTURAL RESEARCH

VOL. 74 WASHINGTON 25, D. C., FEBRUARY 1, 1947 No. 3

PROGENY TESTS OF ASPARAGUS PLANTS[1]

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INTRODUCTION

The Washington strains of asparagus (*Asparagus officinalis* L.) were introduced by Norton (*6*)[2] as the F_2 progenies of individual plants. Presumably they were more or less heterozygous for vigor, and the original variability has probably not been reduced under the usual methods of seed propagation. Currence and Richardson (*2*) found that yields of individual plants on a uniform area of soil gave standard errors in percent of the mean of 79.5, 48.5, and 44.6 for the first, second, and third years of cutting. John Baer tomato plants, under somewhat similar conditions[3] have given a standard error of 25 percent of the mean for one year's yield. In the light of these facts it appears that inherited differences in vigor may exist between asparagus plants, and the important problem of identifying the better parental material and eliminating so far as possible the unproductive germ plasm faces the breeder. Superior breeding plants after being identified can be utilized in a number of ways to produce better seed than that from uncontrolled pollination. This paper presents the results of a study to obtain information regarding the efficiency of several methods of selecting and testing parental plants with some emphasis on the extent of improvement that may be obtained by these methods. The dioecious nature of the plants and the possibility of increasing individuals by asexual propagation make asparagus improvement problems somewhat unique among those of cultivated plants.

MATERIAL AND METHODS

A planting of 1-year-old roots of a commercial strain of the Mary Washington variety was made in 1931, and harvesting records on single plants began in 1933. In 1937 open-pollinated seed was saved individually from a number of pistillate plants taken at random. Seeds of these were started in randomized block plantings in the greenhouse in the fall of 1937. In April of the following year the young seedlings were dug and a number of plants from each replicate were

[1] Received for publication April 2, 1945. Paper No. 2216 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 76.

[3] Unpublished data.

weighed to obtain the comparative growth rates of the strains. Additional samples of the same strains were planted in the field at Grand Rapids, Minn. Facilities limited the size of the planting so that two plant plots were used with six replicates of each strain. Yield records were taken on this planting in 1940 and 1941. Also a few pistillate plants were crossed in pairs with a number of staminate ones. The pollinations were so designed that several combinations would be made for each of the parents. Progenies were started and transplanted in a test of six replicates in a balanced incomplete block arrangement. Harvests were made in 1941 and 1942. To compare the parent plants a test was started by dividing the crowns to form as many plants as possible. These clonal lines of parents were planted in randomized blocks, each clone being placed in as many blocks as the crown divisions permitted. The length of the harvesting period was approximately 6 weeks for each year. Spears were cut when they reached an estimated length of 6 inches or more. No trimming was done on the spears after harvest.

RESULTS

SELECTING FOR YIELD

The yields from the open-pollinated seed of individual plants are of special interest if the pollen may be assumed to have been a mixture from several staminate plants in the planting. The progenies from such seeds would be somewhat comparable to those of top crosses used in corn breeding as a general test for the combining ability of inbred lines. The use of open-pollinated seed may also be comparable to the polycross method suggested by Tysdal and Kiesselbach (*10*) for use with alfalfa, since the crosses are made without previous inbreeding. It may seem to be taking too much for granted to assume that the female plants were pollinated by pollen from several males rather than from only a few adjacent male plants. It has, however, been found that in a block of female plants consisting of three solid rows the center row consistently produces a crop of seed comparable to that produced on the outside rows which are adjacent to rows with a random distribution of male and female plants. In this instance, plenty of pollen has been transported across at least one row and this fact suggests that quite a mixture of pollen parents might occur when open-pollinated seed is saved from female plants growing in a random assortment of the two sexes. Therefore, the yields of the progenies are presented in table 1 as a comparison of the pistillate parents for breeding value and to identify different strains.

The data show significant differences among the progenies, which indicate that the female parents differed in their ability to produce high-yielding sexual progenies. The extent of the differences is apparent from the mean yields of the 19 progenies shown in table 1. Since the extreme between means is 0.98 ton per acre, with 0.385 being significant, it seems that advantage would be gained in commercial growing if seeds were obtained only from the better pistillate parents. The plants 3–5, 2–39, 5–11, 2–20, and 6–29 may be considered as 5 promising pistillate parents for breeding vigorous progenies. This type of test, if of sufficient extent might be helpful in identifying superior female parents for commercial production of seed or for

TABLE 1.—*Yield of pistillate asparagus plants and vigor of their open-pollinated progenies as young seedlings and as producing strains under field conditions*

Pistillate plant	4-year yield of pistillate plant	Mean seedling size of progenies	Calculated acre yields of progenies for 2 years
	Ounces	*Grams*	*Tons*
2-9	59.65	14.75	0.968
2-11	78.14	24.64	.971
2-20	71.02	24.72	1.206
2-35	23.95	33.75	.955
2-39	97.02	23.21	1.424
3-5	75.18	30.12	1.514
3-28	71.85	13.44	.678
3-34	25.28	22.50	.842
4-1	99.17	19.25	1.114
4-4	44.71	22.75	.748
4-15	64.40	19.25	.953
4-38	70.47	20.66	1.071
5-6	64.91	19.00	.536
5-11	54.00	13.69	1.256
5-41	54.10	21.00	.704
6-2	54.06	18.00	.867
6-6	84.12	19.00	.634
6-21	55.74	26.79	.838
6-29	47.51	29.17	1.226
Mean	62.91		.974
Significant difference			.385

further improvement work. If the 8 parent plants that were distinctly higher in yield than the mean of the 19 parents are considered as a group, it will be found that their progenies produced a crop approximately 10 percent larger than the average of all progenies. Thus, a 10-percent increase with this material would have been obtained by selecting these superior female phenotypes. It is also possible to obtain some information as to the effect of making a further selection at the time the progenies were in the seedling stage. If the 8 strains had been reduced to half that number on the basis of seedling size, the 4 remaining progenies with a seedling size greater than the mean of the 8 would be 2–11, 2–20, 2–39, and 3–5. One of these was only average in performance but the other 3 were superior, 2–39 and 3–5 being the 2 best-yielding lines in the planting. The yields of these 4 lines was 1,279, or an increase of approximately 31 percent over the mean of all strains. This would be a worth-while improvement. Yield tests on asparagus are expensive and difficult to make, so that a means of reducing the number of strains to be tested is of practical interest to the breeder.

The extent of improvement that would have been possible if an additional selection had been based on the comparative yields of the progenies and the lines had again been reduced to one-half should also be noted. Obviously 2–39 and 3–5 would have been chosen with a mean yield of 1.469 tons per acre, which is an increase of about 50 percent over the mean of 0.974 for all strains. From these results it is apparent that the essential points in such a selection program would be to save open-pollinated seeds from phenotypically superior pistillate plants and retain those that produce the largest seedlings. The field test would be the final progeny test and this would eliminate all except the best of the lines. It would then be possible to transplant and vegetatively increase the desired plants of the original ones selected. Such superior female plants if isolated with superior or even

average male plants should produce seed of greater productivity than the material from which it was selected.

Strains resulting from controlled pollinations of several different combinations of parental plants offer means of making a more satisfactory study of yield genotypes. From such pollinations definite combinations can be compared and information gained on the breeding value of both pistillate and staminate plants. The data in table 2 show yields and spear sizes of parent plants for the year 1942. The data are for one year on vegetatively propagated plants with various numbers of vegetative progeny from different plants. These were planted in randomized blocks in 1938 with the number of replicates shown as number of plants in test. There is wide variation within the vegetative lines which limits conclusive statements. The results on differences between the phenotypes of the parents are therefore considered as preliminary until further records are available. The standard errors were calculated as the variance within lines. The value of F for yields of pistillate plants was 2.47, the 5-percent point being 2.64. The value of F slightly exceded the 5-percent point for spear size of pistillate parents. For the staminate parents the F value was not significant for either yield or spear size. The male parent plant which will appear later in the results as 3–9 was not represented with the asexual progenies because it was being used in other tests and could not be moved when the crowns were divided.

TABLE 2.—*Yield and spear size of vegetative lines of asparagus plants used in various combinations as parents of test progenies, 1942*

Pistillate plants				Staminate plants			
Identity of parent	Plants in Test	Yield per plant	Spear size	Identity of parent	Plants in test	Yield per plant	Spear size
	Number	*Ounce*	*Ounce*		*Number*	*Ounce*	*Ounce*
5–10	4	25.25± 7.49	2.17±0.232	3–16	6	19.50±4.34	1.72±0.132
5–21	2	27.00±10.60	2.05± .328	2–12	6	20.83±4.34	1.58± .132
2–18	6	40.00± 6.12	1.03± .189	5–32	2	13.00±7.52	1.40± .229
4–5	7	12.43± 5.67	1.34± .175	4–17	9	27.11±3.54	1.42± .108
3–2	8	32.80± 6.70	1.42± .207	5–2	4	14.75±5.32	1.65± .162
2–35	5	31.80± 6.70	1.48± .207	2–3	2	10.00±7.52	1.70± .229

The progeny tests contained 31 progenies which were compared for yield, sex ratios, and spear size. Twenty-four strains composed the progeny from all possible combinations of 6 male plants and 4 female plants. Since this arrangement places parental plants of each sex on a comparable basis, the additional 7 lines are omitted from the yields shown in table 3. As previously stated, the strains were tested under field conditions for 2 years in a balanced incomplete block arrangement. The analysis of variance gives an F value of 4.0, which is definitely higher than the 1.85 required at the 1-percent point. It is obvious therefore that there were differences between the strains in the weight of asparagus produced by them during the 1941 and 1942 seasons. Table 3 shows the comparative yields on the basis of tons per acre with the 2 seasons' records combined. The means for progenies of each parent plant are shown in the last column of table 3 and the means of individual crosses in the body of the table. It is evident that the female plant 5–21 was superior to 5–10 and probably to 2–18,

with a t value of 1.89 for the latter comparison; 4–5 also was superior to 5–10 but was not significantly better than 2–18 or significantly poorer than 5–21. If it were desired to carry on one of these plants, it seems clear that the breeder would choose 5–21 as of most promise, since it is not inferior to the other three and is definitely superior to one of them.

TABLE 3.—*Yield* [1] *in tons per acre of asparagus progenies from various combinations of pistillate and staminate parents; grown in 6 replicates of 3 plants each*

Pistillate parent	Staminate parent						Mean [3]
	3–16	2–12	5–32	4–17	3–9	5–2	
5–10	3.650	3.902	2.612	3.778	2.901	3.742	3.431
5–21	4.433	4.035	3.734	4.143	4.828	4.732	4.318
2–18	4.493	3.884	3.790	3.954	4.366	3.408	3.989
4–5	3.879	4.251	3.334	3.631	4.470	5.446	4.169
Mean [2]	4.114	4.018	3.368	3.877	4.141	4.332	

[1] Significant difference=0.736.
[2] Significant difference=0.350.
[3] Significant difference=0.428.

Of the staminate plants 5–2 was slightly higher than any other with four crosses averaged. The differences are statistically significant only when 5–2 is compared with 5–32 and 4–17; 3–9 also exceeded 5–32 by a significant difference, but did not produce significantly higher yielding progenies than any of the other male parents.

It is possible that individual parent plants react quite differently in different combinations. The interaction, females × males, when tested by the standard error, is significant in some instances. As an illustration of this the progenies from females 2–18 and 4–5 with the males 3–16 and 5–2 may be cited; 2–18 × 3–16 is definitely a good progeny, but 4–5 × 3–16 gives odds of about 15:1 that it is lower in yield. The combination 4–5 × 5–2 was the highest yielding of any combination and 2–18 × 5–2 was inferior in yield. The difference between the differences in tons per acre is 2.551 ± 0.612. Thus it is apparent that the pistillate plant 2–18 forms a favorable combination with 3–16 but a rather poor one with 5–2 and the pistillate plant 4–5 gives the opposite results with the two staminate plants. The results of these tests suggest that individual combinations of parental plants require consideration in breeding asparagus for increased production, and a method of using the best combinations for producing commercial seed would be valuable. With the exception of 5–32 all parent plants shown in table 3 were selected for superior yield. It is therefore impossible to make a completely satisfactory estimate of the degree of improvement that might be expected from seed of outstanding combinations. The highest yielding combination compared with the mean of 5–32 may provide a rough approximation. Inasmuch as 5–32 was an inferior phenotype and the females paired with it were superior ones the mean yield of the four combinations may not be greatly different from what would be expected from seed harvested from a commercial source. If this mean yield is compared with 4–5 × 5–2 the difference is 2.078 tons per acre, or a possible increase of 61 percent due to the favorable combination.

The correlation coefficient between the two variables, mean yield of the two parents and their progeny yields, was 0.259 which does not approach the level of significance for the number of observations made. The parental yields were based on the data shown in table 2. The average of two parents was obtained by averaging the yield of the male and female parent of the particular cross. The two highest yielding parents in the test were 2–18 and 4–17 for the pistillate and staminate, respectively, but the progeny of this combination was not outstanding in yield. It was exceeded by a number of crosses and significantly so by a few. Furthermore, one of the best yielding progenies in the test was that of 4–5 × 5–2. Both of these parents would be considered low in yield as indicated by table 2, but in the production of high-yielding progenies they rank high. Because of the high standard errors of the parental clones, the information obtained from these crosses is not entirely satisfactory, but it does suggest that there may not be sufficient correlation between the vigor of parents and the yield of progenies to give special emphasis to the former in selecting for greater yield. The interaction between genes brought together seems to be of most significance, although, generally speaking, vigorous selections may have better combining ability than weaker ones. Since an adequate number of high-yielding parent plants will normally be available for breeding work, it would seem advisable to use only the most productive for progeny testing and by the progeny test to determine which of them are desired for further use.

RATIO OF PISTILLATE TO STAMINATE PLANTS

Robbins and Jones (*8*, *9*) have demonstrated that male plants are more productive than female. Since a disproportionate ratio of the sexes might therefore affect the comparative yields of the strains, it is of interest to compare the various progenies for the number of male plants in each. Table 4 shows the numbers of the two sexes produced by the various pairs of parents. The data on sex ratios were recorded after all plants had flowered. In addition to those in the

TABLE 4.—*Sex ratios of 31 asparagus progenies from the indicated combinations of parental plants*

Pistillate parent	Sex of progeny	Staminate parent							Open-pollinated	Total
		3–16	2–12	5–32	4–17	3–9	5–2	2–3		
5–10	Male	12	12	15	13	14	8	9	.....	83
	Female	10	[1] 4	9	11	9	16	9	.....	68
5–21	Male	14	8	12	8	17	13	.....	.....	72
	Female	10	12	11	14	[1] 6	10	.....	.....	63
2–18	Male	15	12	19	9	14	13	.....	.....	82
	Female	[1] 6	12	20	11	8	10	.....	.....	67
4–5	Male	10	8	12	11	14	17	.....	.....	72
	Female	14	14	12	13	10	[1] 7	.....	.....	70
3–2	Male	.....	.....	8	7	.....	12	.....	.....	27
	Female	.....	.....	10	10	.....	5	.....	.....	25
2–35	Male	.....	.....	8	.....	.....	9	.....	.....	17
	Female	.....	.....	10	.....	.....	8	.....	.....	18
L–43	Male	.....	.....	.....	.....	.....	.....	.....	9	9
	Female	.....	.....	.....	.....	.....	.....	.....	7	7
Total	Male	51	40	74	48	59	72	9	9	362
	Female	40	42	72	59	[2] 33	56	9	7	318

[1] *P* less than 0.05.
[2] *P* less than 0.01.

test planting, some extras were grown in guard rows. In four individual progenies the deviation from the 1:1 ratio was great enough to be significant by the χ^2 test, suggesting a trend toward excess male plants. However, there are a few of the progenies having excess females which also approach significance.

Totals for parent plants show that only one of them 3–9, had progenies deviating significantly from the theoretical ratio. This also is an excess of male plants and probably in numbers sufficient to indicate that strains with excess male plants are possible as an asparagus-breeding objective. Such strains would have the advantage of containing fewer of the less productive female plants. The data on total progenies such as that of 5–10, 2–18, and 5–2 where the deviations are large but do not give 20 to 1 odds are considered as inconclusive. An additional planting of open-pollinated seed from 2–18 was grown. Combined with the data shown in table 4, the totals are 77 female: 88 male plants with a P value only slightly greater than 0.5. The information available suggests that 3–9 is the only plant tested which can be considered as producing with some consistency an excess of staminate plants in the progeny. Other progenies now being grown from 3–9 contain 40 female: 69 male. The total numbers therefore are 73 female: 128 male, a highly significant deviation from the 1:1 ratio.

Rick and Hanna (*7, p. 712*) suggest "partial sex linkage of some lethal factor" as a genetic explanation of excess male plants in certain strains. Since their data showed that sex in asparagus is controlled by a single gene, occasional crossover types would be expected between the sex gene and the partially linked lethal. In the event of crossing over, there should occur elimination of the male sex. Also it would be essential for the lethal gene to be incompletely dominant since a completely dominant lethal immediately eliminates itself from a population. To explain the data on progenies of 3–9 an incompletely dominant lethal linked with the recessive female-producing gene might be postulated. Incomplete or partial dominance would account for the parent being in existence since this allows for some survival. At the same time, such dominance would account for the uniform elimination of a part of one sex or the other which seems to occur in the progenies of 3–9. It is also necessary to postulate that all the female parents were normal or recessive for the lethal gene and that approximately 33 percent crossing over occurred between the male gene and the lethal. Thus, 67 percent of the female-producing gametes would be eliminated and 33 percent of the male ones. This would tend to give the approximate ratio observed if some few male gametes with the lethal gene survive to give slightly more than one-third males.

Although the numbers are too small to furnish conclusive results, some interesting trends are suggested by crosses other than those involving the 3–9 parent. Among these are the three combinations or crosses that gave significant deviations from the 1:1 ratio, all excess plants being males. None of the deviations for excess females reached significance though a few approached it. Because of the nature of the combinations that gave the significant deviations from the 1:1 ratio, it is suggested that the interaction of genes from the

two parents may effect the elimination or survival of one or both of the sexes and so bring about the disproportionate sex ratios observed.

Because of the possibility that some of the yield differences shown in tables 1 and 3 might have resulted from variations in the sex ratios of the different crosses, correlations between number of male plants and yield between and within the 31 strains were calculated. Within strains there was a very low positive relation, but between strains the coefficient of correlation was only 0.096. From this it is thought that the factors which affected the yield of the strains tested were generally independent of the sex ratios.

SPEAR SIZE

Producers of asparagus generally prefer spears of large diameter; hence the possibilities in breeding for uniformly large spears are of interest. In 1942 a large number of spears were taken from each plot of the balanced incomplete block planting. The diameter of each spear was measured at the thickest part and the diameter means for the plots were calculated. These means have been used to compare the spear sizes of the various progenies, to compare the diameters with the weights of spears, and to test the relation of the spear size of parents with that of their progenies by means of covariance.

The spear size of available parent plants based on average weight in ounces are shown in table 2. The female plants differed significantly as judged by the F value; the F value for the male plants was not statistically significant.

TABLE 5.—*Spear diameter of strains of asparagus in centimeters and the average spear diameter of certain pairs of parents*

Pistillate parent	Generation	Staminate parent							Mixture	Mean of progenies and parents
		3–16	2–12	5–32	4–17	3–9	5–2	2–3		
5–10	Progeny	1.39	1.46	1.17	1.21	1.18	1.24	1.10		1.25
	Parents	1.95	1.88	1.79	1.80		1.91			1.86
5–21	Progeny	1.29	1.67	1.33	1.43	1.28	1.28			1.38
	Parents	1.88	1.82	1.72	1.74		1.85			1.80
2–18	Progeny	1.21	1.14	1.03	1.11	1.19	1.08			1.13
	Parents	1.37	1.30	1.22	1.23		1.34			1.29
4–5	Progeny	1.12	1.22	1.11	1.23	1.29	1.07			1.17
	Parents	1.53	1.46	1.37	1.38		1.50			1.45
3–2	Progeny			1.10	1.30		1.05			1.15
2–35	do			1.04			1.05			1.05
L–43	do								1.15	1.15
Mean	Progeny	1.25	1.37	1.13	1.26	1.24	1.13	1.10	1.15	
	Parents	1.68	1.62	1.53	1.54		1.65			

Among the progenies some produced the large diameter usually desired. The data in table 5, in which spear size of progenies is compared with that of the average of the parents, suggest that this character may be determined by the phenotype of the parent. The cross which gave the greatest spear diameter was 5–21 × 2–12, with a mean of 1.67 cm. The mean of the parents was 1.82 cm., somewhat near the maximum spear size for pairs of parents. The cross giving the smallest spears was 2–18 × 5–32 with a mean of 1.03 cm. This cross was from parents of small size. Although several exceptions must be noted, the data seem to offer some possibilities of selection for spear size based on spear size of parent plants. Inasmuch as the parents were partly se-

lected for large stalks it is probable that practically all these progenies have larger than average spears. Two exceptions are the progenies of L–43 and those of the staminate parent 5–32. The latter was a small, weak plant and the former progeny was produced by a mixture of pollen, so that progenies of these come from crosses in which only one parent was superior and therefore they would be expected to exceed the average to a less extent than progenies having both parents selected. Thus it may be assumed that any population in this test exceeding a spear diameter of about 1.10 cm. would be greater than average commercial stock in size of spears produced.

The covariance analysis for testing the data for correlation between spear weight and spear diameter is shown in table 6. Since it is less arduous to count spears and weigh them in bulk than to measure diameter, it is of interest that the correlation coefficient gives a high value, showing that diameter may be predicted fairly accurately from spear weight.

From the standpoint of general desirability the cross 4–5 × 3–9 appears to be one of the best. It gave a fairly good yield with satisfactory size of spears. Other crosses produced larger spears and some were more productive but it is doubtful whether any were superior in both respects.

TABLE 6.—*Analysis of covariance for spear weight and diameter of 31 strains of asparagus*

Source of variation	Degrees of freedom	Sum of squares or product			Mean square or product				Regression of diameter on weight
		Diameter	Products	Weight	Diameter	Products	Weight	r	
Blocks	30	2.258	1.745	2.203	0.075	0.058	0.073	0.782	0.792
Progenies	30	3.123	3.239	4.220	.104	.108	.141	.892	.767
Error	125	6.255	1.130	2.403	.050	.009	.019	.292	.470
Total	185	11.636	6.114	8.826	.063	.033	.048	.466	.693

DISCUSSION

In an asparagus improvement program the genetic combinations brought together are no doubt important, and indiscriminate isolating of productive plants for seed production does not appear very promising as a means of obtaining superior seed. For male parents it would seem desirable to have progeny tests of several combinations. If in doing this a male plant is found that produces uniformly vigorous progenies, it might be vegetatively divided and grown in a seed plot. Progeny testing of female plants is somewhat easier than that of male plants if use is made of open-pollinated seed. This seed, of course, should be obtained from a planting in which pollen from several different males was utilized. Female plants chosen by this method should then be put in an isolation plot with males that have demonstrated good combining ability. In the planting of such a plot advantage should be taken of the fact that the plants can be vegetatively reproduced. Several more female plants will be needed than males; 1 male plant to about 10 or 12 female plants should be sufficient to provide the necessary pollen. With a rather crude technique a 4-year-old crown has been divided into 20 plants, and an even greater num-

ber might be obtained with proper technique. If such divisions are repeated at intervals, the eventual building up of a fairly adequate supply of parent plants should not prove too difficult.

Tysdal and Kiesselbach (*10*) have called attention to the possibility of using vegetative propagation and self-sterility to obtain commercial seed of alfalfa. This plan provides for the use of the best single cross known and obviates the necessity of inbreeding several generations. Since asparagus is dioecious it would seem feasible to employ somewhat the same method, although the supply of seed produced would be rather small for a good many years. An isolation plot might contain only 1 male clone with 10 or 12 females if they are known to combine well with the staminate plant. Seed from the different female clones would then be single crosses and might be marketed separately as individual single crosses if desired. Starting with 1 staminate and 5 pistillate parents and dividing them every 4 years into 10 plants each, would require 3 divisions or 8 years to build up to 6,000 plants, or roughly the number required to plant an acre at the common spacing of 1.5×5 feet. Since in 12 years 10 acres could be planted, this plan seems rather promising. Manifestly in starting with only 2 parents, 1 staminate and 1 pistillate, the program would be considerably delayed by getting the female clone built up. Because of this about 12 years might be needed to obtain 2 acres of seed-producing plants.

A rather serious limiting factor in this program might be the labor needed to dig and divide a large number of old asparagus crowns, but it is also reasonable to expect that methods could be developed that would reduce the labor required. If resources were adequate the divisions might be grown in a loose soil in containers and then removed by washing. This would preserve the root system and speed up the program. No doubt other time-saving methods would be evolved by workers if this type of improvement were applied.

Prior to 1942 a small beginning was made on increasing promising clones. A few plants of 3–9 and 5–2 are now growing in isolated plots with a few plants each of 4–5 and 2–18. There is no assurance that repeated propagation by crown divisions will be successful, but no serious difficulty has been experienced in getting vigorous plants from the first division.

To produce a large amount of commercial seed, the possibilities of a double cross from outstanding parents should be investigated. In corn-breeding work Jenkins (*5*) and others have demonstrated the importance of making double crosses from the proper single crosses. With four inbred lines of corn there are six possible single crosses and three possible double crosses, but since asparagus is dioecious there are only four possible combinations from four plants. In corn the methods of predicting the performance of double crosses from the performance of single crosses are shown by the work of Jenkins (*5*), Anderson (*1*), Doxtator and Johnson (*3*), Hayes and Johnson (*4*), and others.

It appears quite accurate to estimate double-cross yields from the average of four single crosses that contain neither of the combinations entering into the parentage of the double cross. From the results given in table 3 it would seem that the double cross (5–21 × 5–2) ×

(4–5 × 3–9) should be a good one. These are the most promising parents from the standpoint of breeding behavior for yield as well as for other characters and the single crosses were the two lower yielding of the four possible combinations. It would therefore be expected that this double cross would give a more vigorous population and greater yield than the double cross from the more productive single crosses. By building up clonal lines of the original parents and isolating them in proper combinations, seed of single crosses could be obtained. This seed would then be planted and the two sexes identified. Staminate plants from the one single-cross parent would be put in an isolated planting with pistillate plants from the other single cross. In a second isolation plot the opposite sexes from the two crosses would be grown. Seed produced from these isolated plots would be double crosses and should exhibit considerable hybrid vigor although not as much as the single crosses. Within a few years a large amount of commercial double-cross seed could readily be produced by this method.

Rick and Hanna (7) suggest another approach to the problem of getting productive strains. They outline a plan for breeding an all-male population to take advantage of the greater production by male plants and also to provide the seed producer with monopolistic control of his product. This would seem a very promising line of attack. Although the writer has been unable to find viable seed on male plants, Rick and Hanna imply that there would be no particular difficulty in doing this under the conditions of their experiments. With homozygous male plants available and information on their combining ability as well as on that of the female parents, it should be possible to obtain a very productive strain.

A small beginning toward inbreeding has been made by sib-crossing some of the better strains from known parents and one of foreign origin. It is hoped after several generations of sib-crossing to have inbred strains that, when combined, will show sufficient hybrid vigor to provide a productive single cross for commercial purposes. Considering the favorable results that this method has given with other crops, especially corn, it should be adequately tested for asparagus. The nature of the plant and its perennial habit offer some serious obstacles but also certain advantages. If suitable strains are once obtained and identified for proper combinations it will be comparatively simple to maintain the lines and combine them as desired for commercial seed production. A fact which may have considerable significance in the choice of one or several of the possible methods of asparagus improvement is that by retaining all parental material, the originator would be in a position to maintain a monopoly on the commercial seed and should be able to realize a profit in proportion to the degree of improvement that the material offers over other strains.

SUMMARY

Open-pollinated seed from 19 pistillate asparagus plants was grown and records were taken on seedling size and yields of the 19 strains. The better phenotypes tended to produce better progenies than the poorer ones so that a yield increase of about 10 percent might be expected from the best 8 of the 19 parent plants. If a further selection

had been made at the time of transplanting an increase of about 30 percent would have been obtained. The desirability of making progeny tests was shown by the 2 best lines producing a 50-percent greater crop than the mean of all lines.

Progenies from different combinations of parental plants of both sexes were compared for yield, sex ratio, and spear size. Distinct differences were noted between parents for transmitting yield genes and between single crosses for favorable combinations. It is thought that the combination of dominant yield genes of the parents needs emphasis in the development of high-yielding strains of asparagus as it does for corn and other crops. Yields of parent plants, vegetatively divided, did not seem to bear any outstanding relation to the yields of their progenies either in several combinations or in individual combinations.

Total progenies from one male parent differed significantly from the theoretical sex ratio. All significant deviations were the result of excess males with the disproportionate ratios approaching 1 female : 2 males. However, the number of males in these progenies appeared to exert little effect on the yields of single crosses. Because of a few combinations which gave deviations approaching significance and those which were significant, the possibility is suggested that interaction of genes might eventually be found to be the genetic basis for disproportionate sex ratios.

Average spear size of the paired parents seemed to be a rather poor indication of the spear sizes to be expected in the progenies. By measuring diameters and weighing samples from the different plots it was found that diameter was highly correlated with weight so that weight provides an adequate estimate of spear diameter.

Several methods of breeding asparagus for more productive strains are discussed.

LITERATURE CITED

(1) Anderson, D. C.
1938. The relation between single and double cross yields in corn. Amer. Soc. Agron. Jour. 30: 209–211.

(2) Currence, T. M., and Richardson, A. L.
1938. Asparagus breeding studies. Amer. Soc. Hort. Sci. Proc. (1937) 35: 554–557.

(3) Doxtator, C. W., and Johnson, I. J.
1936. Prediction of double cross yields in corn. Amer. Soc. Agron. Jour. 28: 460–462.

(4) Hayes, H. K., and Johnson, I. J.
1939. The breeding of improved selfed lines of corn. Amer. Soc. Agron. Jour. 31: 710–724.

(5) Jenkins, M. T.
1934. Methods of estimating the performance of double crosses in corn. Amer. Soc. Agron. Jour. 26: 199–204, illus.

(6) Norton, J. B.
1913. Methods used in breeding asparagus for rust resistance. U. S. Dept. Agr. Bur. Plant Indus. Bul. 263, 60 pp., illus.

(7) Rick, C. M., and Hanna, G. C.
1943. Determination of sex in asparagus officinalis L. Amer. Jour. Bot. 30: 711–714.

(8) Robbins, W. W., and Jones, H. A.
1925. Secondary sex characters in asparagus officinalis L. Hilgardia 1: [183]–202, illus.

(9) ———, and Jones, H. A.
1929. Further studies on sex in asparagus. Amer. Soc. Hort. Sci. Proc. (1928) 25: 13–16, illus.

(10) Tysdal, H. M., and Kiesselbach, T. A.
1944. Hybrid alfalfa. Amer. Soc. Agron. Jour. 36: 649–667.

THE QUANTITATIVE ESTIMATION OF HEMICELLULOSES BY DIRECT ISOLATION[1]

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INTRODUCTION

The various methods described in the literature for the quantitative estimation of hemicelluloses were reviewed in an earlier paper (*5*) [2]. It was pointed out that the analytical methods, based either on the determination of the quantity of reducing sugars produced when these carbohydrate complexes are subjected to acid hydrolysis or on the determination of the percentage of furfural, afford results that are difficult to evaluate in terms of actual quantity of hemicellulose in the material under examination.

Preece (*6*) has introduced a method for the quantitative estimation of hemicelluloses based on the isolation and weighing of the hemicelluloses. According to the Preece procedure, the sample (20-25 gm.) is given a preliminary extraction first with a hot 0.5-percent aqueous ammonium oxalate solution, then with a 1-percent boiling sodium hydroxide solution in 50-percent ethanol, and finally with a 50-percent ethanol solution. The residual material is then repeatedly extracted, at room temperature (25°-30° C.), with a 4-percent aqueous sodium hydroxide solution, until a portion of the extract no longer gives a precipitate when neutralized with acetic acid and diluted with an equal volume of acetone. The combined alkaline extract is then rendered acid by the addition first of glacial acetic acid and then of acetone equal in volume to the acidified solution. The precipitate is filtered, dried in vacuo and the weight of the moisture- and ash-free product is determined. The result thus obtained is calculated as percentage of free hemicellulose. The material remaining from the extraction with the alkali solution is further extracted with 4-percent boiling aqueous sodium hydroxide solution, and the product is isolated as previously described. The result is reported as percentage of combined hemicellulose.

So far as the writers are aware, no attempt has yet been made to examine the Preece method critically. In a slightly modified form, the method was used by Buston (*2*, *3*) and by Bennett (*1*) in their studies of the cell-wall constituents of plants. However, when the plant material is extracted according to the Preece procedure, not only are the hemicelluloses removed but some of the lignin also, with the result that both the free and combined hemicellulose fractions of Preece are contaminated with lignin. Moreover, when the extraction

[1] Received for publication July 11, 1945.

[2] Italic numbers in parentheses refer to Literature Cited, p. 85.

of combined hemicellulose is made with 4-percent aqueous boiling sodium hydroxide solution, considerable degradation of the hemicelluloses is likely to occur.

In the present paper, a procedure is described for the quantitative estimation of hemicelluloses in straw, which consists essentially of two steps: (1) the isolation of the holocellulose from a sample which has previously been freed of alcohol-benzene extractives and pectic substances, and (2) the isolation of the hemicellulose from the holocellulose. The residual material is weighed to determine the total amount of material extractable with dilute sodium hydroxide solution. This method for the quantitative estimation of hemicelluloses is believed to have definite advantages over the other methods described in the literature. It is realized, however, that no method entirely free from criticism can be developed with our present limited knowledge of the chemistry of hemicelluloses.

METHODS OF EXPERIMENTATION

APPARATUS

The apparatus shown in figure 1 was found to be very useful in determining the percentages of pectic substances and holocellulose. It consists of a fritted-glass crucible, *B*, 5½ inches tall and 1¾ inches in diameter, the lower end of which was rimmed to fit the rubber stopper

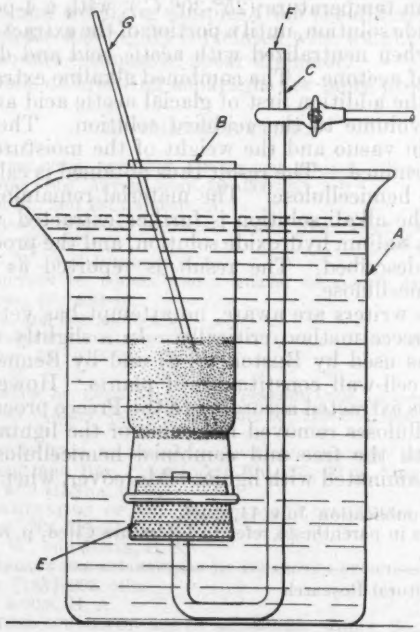

FIGURE 1.—Apparatus for determining pectic substances and holocellulose.

E. The fritted-glass disk of the crucible was made from ground glass, 80-100 mesh. Through an opening in the stopper *E* the tube *C* is passed and any solvent in *B* can be removed by applying suction at *F*. The contents of *B* are mixed with the glass rod *G*. With this apparatus it was possible to make the two determinations with the minimum manipulation and without the necessity of transferring the sample from one container to another.

EXTRACTION OF THE SAMPLE

The determination of the 1:2 alcohol-benzene extractives in the plant material under investigation was carried out as follows: A 5-gm. sample was placed in the crucible *B* which was then inserted into a Soxhlet extraction apparatus and the extraction was allowed to proceed for 30 hours. Then the crucible was taken out, and as much as possible of the adhering solvent was removed by suction. The crucible and its contents, next dried in vacuo at 60° C., were finally weighed.

The extraction of pectic substances (and other constituents soluble in the 0.5-percent aqueous ammonium oxalate solution) was carried out as follows: To the crucible *B*, the rubber stopper *E* and the tube *C* were attached. The crucible was nearly filled with hot (85° C.) 0.5-percent aqueous ammonium oxalate solution and the entire assembly was placed in beaker *A* which was filled with water and maintained at a temperature of 85°. The crucible was allowed to remain in the beaker for 1 to 1½ hours, while the plant material and ammonium oxalate solution were stirred from time to time with the glass rod *G*. After each extraction period, the solution in *B* was removed by applying suction at *F* and the digestion with fresh 0. 5-percent ammonium oxalate solution was then repeated. After the removal of all pectic substances (determined by adding to a portion of the ammonium oxalate extract four times its volume of ethanol, previously acidified with hydrochloric acid, and then noting whether any precipitate separated out), the residual plant material was washed with water until free of ammonium oxalate, then washed with 95-percent ethanol, dried in vacuo at 60°, and weighed.

The holocellulose in the plant material, which had been successively extracted with alcohol-benzene and ammonium oxalate solutions, was next determined, a modification of the procedure recommended by Ritter and his coworkers (*4*, *7*) being followed. A somewhat different procedure was used for the chlorination of the sample, and the washing with cold water after chlorination was eliminated.

The chlorination was carried out in a special apparatus, illustrated in figure 2, consisting of a glass jar *B* provided with a glass cover *D*, which had been ground to fit tightly to the top of *B*. Through the opening in *D*, the one-holed rubber stopper *O* was inserted. The glass tube passing through the stopper *O* was connected by means of a rubber tube provided with a Hofmann clamp, *E*, to the T tube *P*. *P* in turn was connected by means of suitable tubes, as shown in the drawing, to the bottles *H* and *J* and to the one-liter suction flask *N* containing dilute sodium hydroxide solution for the absorption of chlorine. The various rubber tubes were provided with the clamps *F*, *G*, *I*, *K*, *L*, and *M*. *H* was almost filled with a saturated solution of sodium chloride. Chlorine from a tank entered the system at *Q*. By closing

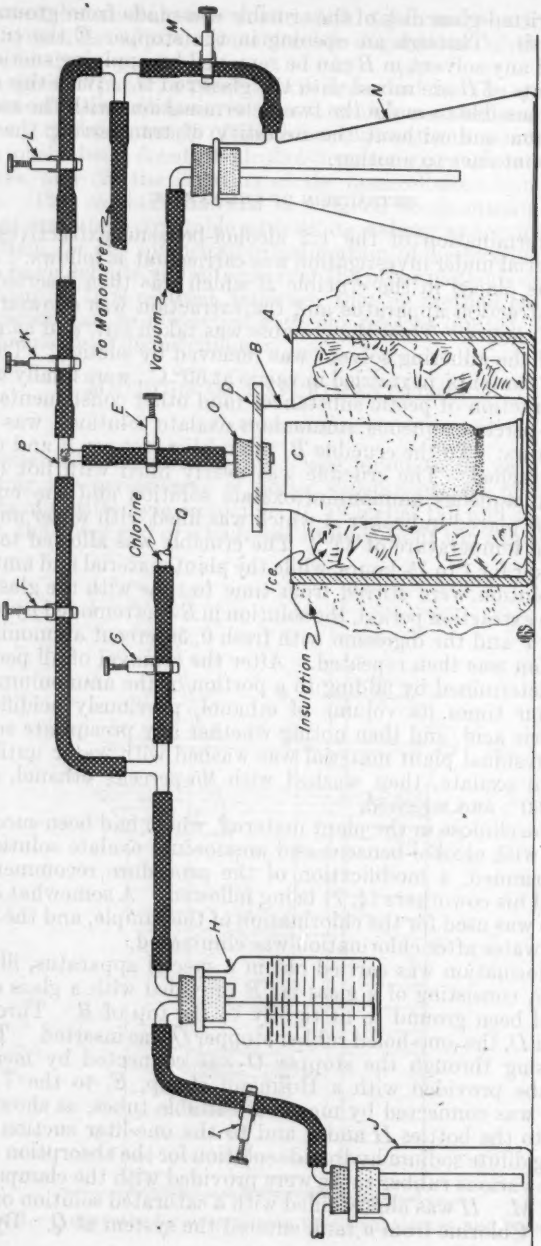

FIGURE 2.—Chlorination apparatus.

the clamp *F*, and opening the clamps *G* and *I*, chlorine from *Q* could be led into *H* and the salt solution driven over into *J*. *B* was placed in an insulated jar *A* and the space between *A* and *B* was filled with crushed ice. The sample to be chlorinated was contained in the fritted-glass crucible *C*.

ISOLATION OF HOLOCELLULOSE FROM THE SAMPLE

The determination of the holocellulose was carried out as follows: The plant material in figure 2, *C*, which had been successively extracted with 1:2 alcohol-benzene solution and with 0. 5-percent ammonium oxalate solution was moistened with about 12 ml. of distilled water and placed in the chlorination chamber *B*. *B* was placed in *A* and the latter was filled with crushed ice. *D* was placed on the top of *B* and *O* was replaced with a thermometer provided with a suitable stopper. The thermometer was inserted to extend within an inch of the sample in *C*. When the temperature in *C* was 15° C., or below, the thermometer was removed and *O* was inserted into the opening of *D*. With *F* closed and *E*, *K*, and *L* open, the system was evacuated by opening *M*.

By means of a monometer not shown in the drawing, it was determined whether the system was gastight before the chlorine was permitted to enter. With *K*, *L*, and *M* closed and *G* and *I* open, chlorine from a tank was first passed through a gas wash bottle containing water and was then allowed to enter at *Q* until about one-half of the salt solution in *H* was displaced and forced into *J*. *F* was slowly opened, while the flow of chlorine entering at *Q* was increased. The valve on the chlorine tank and *F* were so regulated that the level of the liquid in *H* remained about the same. When the pressure of the chlorine in *B* was approximately atmospheric, *H* was allowed to fill with chlorine. Next the valve of the chlorine tank and the clamp *G* were closed. Then the level of the liquid in *H* rose slowly, owing to the absorption of the chlorine by the sample in *C*. When the absorption of chlorine had ceased, *F* was closed. *C* was removed from the chlorinating chamber *B*; 95-percent ethanol was added to *C*; and the sample and the ethanol were thoroughly mixed with a glass rod. The ethanol was removed with suction and the washing with this solvent was repeated. To *C* was then added a 3-percent ethanolamine solution in 95-percent ethanol and the contents were stirred with the glass rod. After it had stood for 2 minutes, the solution in *C* was drawn off with the aid of a suction flask. The treatment with the ethanolamine solution was repeated. The reaction product was washed twice with ethanol; once with ether; and was then freed of solvent with the aid of the suction pump.

The sample in the crucible *C* was moistened again with about 12 ml. of distilled water. Then the chlorination and the subsequent treatments with ethanol, ethanolamine solution, ethanol, and ether were repeated. This process of alternate chlorination and extraction was repeated until the chlorinated sample no longer produced a coloration with ethanolamine solution. The crucible *C* and its contents were dried in vacuo at 60° C., weighed, and the percentage of holocellulose in the sample was calculated.

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ISOLATION OF HEMICELLULOSES FROM HOLOCELLULOSE

The experiments hereinafter described were undertaken for the purpose of determining the optimum conditions for extracting the hemicelluloses from holocellulose. The following procedure unless specifically indicated otherwise, was used in these experiments:

To 1 gm. of holocellulose, contained in a 200-ml. Erlenmeyer flask, a specified volume of 4-percent aqueous sodium hydroxide solution was added, the flask was stoppered, and the mixture was allowed to digest at room temperature (25°–30° C.) for a specified period. From time to time the reaction mixture was shaken manually. At the end of the allotted interval, the reaction mixture was filtered on a cloth through a small Hirsch funnel; the residual material was washed with 15 ml. of water; and the washings were added to the main alkaline filtrate. To the filtrate 3 volumes of ethanol were added, the solution was made slightly acid with acetic acid, and the mixture was allowed to stand overnight at room temperature. The supernatant liquid was then drawn off, and the hemicellulose material was filtered in a weighed sintered glass crucible of fine porosity and washed with 100 ml. of an aqueous alcohloic solution (made by adding 25 ml. of water and 1 ml. of glacial acetic acid to 75 ml. of 95-percent ethanol). It was next washed successively with 95-percent ethanol, absolute ethanol, and ether; then dried in vacuo at 60° and weighed.

An experiment was first carried out for the purpose of determining the rate of extraction of the hemicelluloses from holocellulose. To 1 gm. of holocellulose, 100 ml. of a 4-percent aqueous sodium hydroxide solution was added. After the reaction mixture had been allowed to stand at room temperature for a given period, it was filtered, and the hemicelluloses were isolated as described above. The residual material resulting from the extraction of the hemicelluloses with the alkaline solution was subjected to successive extractions, at room temperature, with aqueous 4-percent sodium hydroxide solution. The hemicelluloses obtained in each extraction were determined separately.

TABLE 1.—*Successive extraction of hemicelluloses from 1 gm. of wheat straw holocellulose, at room temperature, during a 2-hour period, with 100 ml. of 4-percent aqueous sodium hydroxide solution*

Order of extraction	Weight of hemicelluloses	Weight of residue	Loss or gain
	Gram	*Gram*	*Percent*
First	0.3227		
Second	.0128		
Third	.0066		
Fourth	.0034		
Fifth	0	0.5770	−7.75
Sixth [1]	.0486	.4862	−4.22
Seventh [1]	.0418	.4454	+.10

[1] The extraction of the residual material was made at 100° C. during a period of 1 hour.

These operations were continued until a portion of the alkaline extract, on the addition of 3 volumes of ethanol and acidulation with acetic acid, gave no precipitate. The residual material after being washed with alcohol and ether and dried was weighed and then extracted twice at 100° C. with a 4-percent aqueous sodium hydroxide solution, each extraction lasting 1 hour. The results obtained are

recorded in table 1. (All results on the yield of hemicelluloses represent the average of at least two determinations.)

From the results shown in table 1 it can be seen that even in the alkaline extract from the third successive extraction of wheat straw holocellulose, some hemicellulose equivalent to nearly 0.7 percent of the starting material was obtained. It appears, then, that the removal of the hemicelluloses from wheat straw holocellulose, at room temperature, proceeds rather slowly, since four successive extractions were required to remove all the readily available hemicelluloses. The subsequent extraction of the residual material at 100° C. resulted apparently in a progressive degradation of the cellulose.

In the hope that the time required for separating the hemicelluloses from the holocellulose might be reduced, a series of extraction experiments at room temperature was carried out under the same conditions as those recorded in table 1, except that the extraction period was reduced to 1 hour. The results obtained are recorded in table 2.

TABLE 2.—*Successive extraction of hemicelluloses from 1 gm. of wheat straw holocellulose, at room temperature, during a period of 1 hour, with 100 ml. of 4-percent aqueous sodium hydroxide solution*

Order of extraction	Weight of hemicelluloses	Weight of residue	Loss
	Gram	*Gram*	*Percent*
First	0.3192		
Second	.0099		
Third	.0075	0.5869	7.65
Fourth	0		

It will be observed from a comparison of tables 1 and 2 that during the 1-hour extraction period the total yield of hemicelluloses (0.3366 gm.) was somewhat less than that obtained during the 2-hour period (0.3455 gm.). The loss, or difference between the starting material and the sum of the weights of the hemicelluloses and residue, was about the same in both series of experiments.

In a third series of experiments the holocellulose was exhaustively extracted, first with a 2-percent aqueous sodium hydroxide solution; and then with a 4-percent aqueous sodium hydroxide solution. It was believed that the weaker alkali solution might afford a greater yield of hemicellulose, presumably because of its less drastic action on the hemicelluloses. The results obtained are recorded in table 3.

TABLE 3.—*Successive extraction of hemicelluloses from 1 gm. of wheat straw holocellulose, at room temperature, during a 2-hour period, first with 100 ml. of 2-percent aqueous sodium hydroxide solution and then with 100 ml. of 4-percent solution*

Order of extraction	Concentration of sodium hydroxide solution	Weight of hemicelluloses	Weight of residue	Loss or gain
	Percent	*Gram*	*Gram*	*Percent*
First	2	0.2852		
Second	2	.0024		
Third	2	0	0.6442	−6.82
First	4	.0444		
Second	4	0	.6070	+.72

It will be observed from table 3 that the total amount of hemicelluloses extracted by the 2-percent sodium hydroxide solution was 0.2876 gm. The total extracted, by both concentrations of the solution, was 0.3320 gm. The extraction of the holocellulose first with 2-percent and then with 4-percent aqueous sodium hydroxide solution gave a lower yield of hemicelluloses than the 4-percent extraction shown in table 1, although the loss sustained when the extraction was carried out with 2-percent aqueous sodium hydroxide solution (table 3) was somewhat less than in the experiments recorded in table 1. The small apparent gain obtained when the residual material, which had already been extracted with 2-percent aqueous sodium hydroxide solution, was subjected to extraction with 4-percent aqueous sodium hydroxide solution was undoubtedly due to an experimental error.

Next a series of experiments was carried out which differed from the previous series only in that the extraction was made first with a 1-percent instead of a 2-percent aqueous sodium hydroxide solution. The hemicellulose fraction extracted by the 1-percent solution amounted to 0.2086 gm., whereas that extracted by the 4-percent aqueous sodium hydroxide solution was 0.1197 gm. The total yield of hemicelluloses was 0.3283 gm., somewhat less than the yield obtained in the experiments recorded in table 3. The loss resulting from the extraction operations was approximately the same as that sustained in the previous series of experiments. There appeared to be no advantage in making the extraction with the 1-percent aqueous sodium hydroxide solution.

It may be of interest in this connection to mention the fact that when 1 gm. of wheat straw holocellulose was digested for 2 hours with 100 ml. of distilled water, 0.1128 gm. of hemicellulose was obtained. The loss amounted to 4.28 percent.

DISCUSSION

When the technique described above is followed, no difficulty is experienced in getting consistent checks of the percentage of holocellulose in the sample. It is, of course, conceivable that, even under the relatively mild conditions employed for the isolation of holocellulose, some group or fraction constituting an integral part of the hemicellulose complex may have been split off. However, it is believed that any error arising from this source cannot be appreciable.

The development of a satisfactory method for the quantitative estimation of hemicelluloses in holocellulose is complicated by the fact that the hemicelluloses are a rather ill-defined group of carbohydrate complexes. For the purpose of this investigation, hemicelluloses have been defined as carbohydrate complexes that are extracted from the holocellulose with dilute aqueous sodium hydroxide solution, at room temperature, and that are precipitated from the alkaline extract by the addition of an excess of ethanol. While this definition may be arbitrary to some extent, this would be equally true of any other definition of hemicelluloses that might be proposed.

SUMMARY

A method for the quantative estimation of hemicelluloses in wheat straw is described. It involves first the isolation of holocellulose from a sample which had previously been extracted with a 1:2 alcohol-benzene solution and with a 0.5-percent aqueous ammonium oxalate solution, and then the removal of the hemicelluloses from the holocellulose by extraction, at room temperature, with a 4-percent aqueous sodium hydroxide solution.

The maximum yield of hemicelluloses is obtained when the holocellulose is extracted at room temperature four successive times, for periods of 2 hours each, with a 4-percent aqueous sodium hydroxide solution.

LITERATURE CITED

(1) Bennett, E.
1940. observations on the development of certain cell-wall constituents of forage plants. Plant Physiol. 15: 327–334, illus.

(2) Buston, H. W.
1934. the polyuronide constituents of forage grasses. Jour. Biochem. 28: [1028]–1037.

(3) ———
1935. observations on the nature, distribution and development of certain cell-wall constituents of plants. Jour. Biochem. 29: [196]–218.

(4) Hajny, G. J., and Ritter, G. J.
1941. holocellulose research in cooperation with the technical association of the pulp and paper industry. Paper Trade Jour. 113 (13): 83–87, illus. (Tappi sect., pp. 143–147).

(5) Phillips, M.
1940. the hemicellulose constituents of the nitrogen-free extract. Assoc. Off. Agr. Chem. Jour. 23: 119–126.

(6) Preece, I. A.
1931. studies on hemicelluloses. IV. the proximate analysis of boxwood, and the nature of its furfuraldehyde-yielding constituents. Jour. Biochem. 25: [1304]–1318.

(7) VanBeckum, W. G., and Ritter, G. J.
1937. rapid methods for the determination of holocellulose and cross and bevan cellulose in wood. Paper Trade Jour. 105 (18): 127–130, illus. (Tappi sect., pp. 277–280).

SOME CHEMICAL TREATMENTS AND THEIR INFLUENCE ON DAMPING-OFF, WEED CONTROL, AND WINTER INJURY OF RED PINE SEEDLINGS[1]

By A. J. Riker, *professor*, R. H. Gruenhagen, L. F. Roth, formerly *research assistants, Department of Plant Pathology, Wisconsin Agricultural Experiment Station*, and W. H. Brener, *Manager, Griffith State Nursery*.[2]

INTRODUCTION

The control studies described herein were made because damping-off continues to influence production in Wisconsin forest nurseries. The causal fungi seem to be distributed widely in cultivated soil, but to be relatively scarce in unbroken forest soil (*7*).[3] While serious outbreaks are sporadic, small losses are expected each year. The unpredictable occurrence of epidemic attacks in the various nurseries is a handicap to the sustained production demanded by an extensive planting program.

In Wisconsin damping-off is caused principally by two fungi, *Pythium irregulare* Buisman and *Rhizoctonia solani* Kühn, although seedlings are occasionally damped-off by other micro-organisms. These two fungi induce damping-off with somewhat different symptoms and are active under different circumstances, so that control measures against one may have little effect on the other.

For example, *Pythium* operates in Wisconsin at relatively cool temperature, high humidity, and in neutral to slightly acid soil. In contrast, *Rhizoctonia* is active particularly in relatively warm, dry soil with an acid reaction (*8*, *9*). Nevertheless, control measures need to be effective against both, and they are not especially differentiated in this paper.

Reasonable commercial control has often been secured in Wisconsin by planting on newly cleared forest land, by fall seeding, by covering late spring seeding with sand, and by soil treatments, especially with dilute sulfuric acid. These methods have been used alone or in various combinations. Nurserymen used new land as long as the nurseries were expanding, but, eventually, they reached a limit when they had to replant land that had already grown tree seedlings. Fall seeding has been the more common practice, but sometimes circumstances have required spring planting. Late spring planting

[1] Received for publication August 18, 1945.

[2] The writers are indebted to the Wisconsin Conservation Department and to the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, for cooperation and encouragement in these investigations; to Carl Hartley, F. G. Kilp, Leif Steiro, and S. A. Wilde both for suggestions and for cooperation during the studies herein reported.

[3] Italic numbers in parentheses refer to Literature Cited, p. 95.

with the seed covered by sand is a nursery application of the greenhouse practice common with many kinds of susceptible plants.[4]

Both the sterilizing effect of sulfuric acid and the increased soil acidity prevent damping-off and kill many weed seeds. However, acid treatment is relatively expensive to apply and is harmful to the nurseryman's clothes and skin if improperly handled. There is some possibility of chemical injury to the seedlings, but worst of all (*10*) is the fact that the acid undesirably disturbs the base exchange capacity of the soil.

This paper presents studies aimed at a control of damping-off as efficient as sulfuric acid but at one that will not change conspicuously the nutritional balance in the soil. An abstract covering some of the work has already appeared (*6*).

MATERIALS AND METHODS

In the early stages of the work, many different trials were made in 4- and 6-inch pots in the greenhouse where a number of unpromising materials were eliminated.

The soil employed was Plainfield sand secured from near the location of nursery trials at Wisconsin Rapids in order that the greenhouse studies might be more directly applicable to nursery conditions in central Wisconsin. An analysis of this soil is available (*8*).

Seed from local red pine (*Pinus resinosa* Ait.) was used for the most part, but some observations were also made with seed from Scotch pine (*P. sylvestris* L.) and from Austrian pine (*P. nigra* Arn.).

The fungi used for soil inoculation were *Pythium* and *Rhizoctonia*, as described by Roth and Riker (*8*). Greenhouse pots were planted with a counted number of seeds, usually 25, 50, or 100 seeds per pot, depending primarily upon its size. The different treatments were made with suitable numbers of replications. The soil in some of the trials was steamed for 20 minutes at 15 pounds pressure in 6-inch pots. However, most of the trials had a natural woods soil practically free (*8*) from damping-off fungi.

For those treatments employing seed dusted with a disinfectant, the seed and dust were placed in a drum about 4 inches in diameter and 7 inches long which was provided with 1-inch baffles. The drum was closed and slowly rolled for 10 feet. The seed was removed from the drum, separated from excess dust over a fine mesh screen, and placed in packets prior to planting.

For nursery plantings one could easily treat the seed in grain dusters, such as those in common use (*4*).

The nursery trials were made in three Wisconsin locations. Nurseries 1 and 2 were located on Plainfield sand in central Wisconsin, while No. 3 was on Vilas light sandy loam in northern Wisconsin. Both of these soils had a pH near 5.7, and for some years previous had been employed in growing nursery pine trees. The seed and fungi were similar to those in the greenhouse studies. Where the soil was inoculated, damping-off fungi were introduced from sand-corn-meal cultures grown in mason jars. The fungus and its corn-meal substrate were passed through a quarter-inch wire mesh and worked

[4] The Evergreen Nursery, Sturgeon Bay, Wis., has eliminated its damping-off problem by covering the seed with Lake Michigan sand.

into the surface soil of the seedbed at the rate of 1 pint of culture for 16 square feet.

Dry soil treatments that were strong enough to injure the seed were applied evenly over the surface of the soil, were worked in to a depth of 1.5 inches, and were allowed to stand 3 days before seeding. Seeding was made without disturbing more than this top 1.5 inches of soil, and the calomel treatment was made at this time. The chemical was dusted into the seed trenches at the rate of 0.4 gm. per square foot of plot area.

All of the liquid treatments were applied just after seeding, at the rate of one-half pint per square foot. The liquid-treated beds were then leached with an equal volume of clear water.

All of the seedbeds were covered with wire screen and half shade to prevent injury by rodents, birds, and excessive sunshine. In both the smaller and larger field plantings the various treatments in each replication were distributed by chance.

Records of emergence and damping-off of the small-scale plantings were made at frequent intervals. Records on germination included the entire area planted, but those for damping-off excluded 3 inches marked off around the edge to eliminate marginal effects.

Seedlings which were damped-off and those which were chemically injured were differentiated according to the descriptions of Hartley (*2*, *3*) and Roth and Riker (*7*). All standing seedlings were given a slight brushing pull upward. If they came up, they were counted according to the character of the lesion. If they resisted the pull, they were considered healthy. This gave the benefit of the doubt to seedlings with minor chemical injury.

EXPERIMENTAL RESULTS

GREENHOUSE STUDIES

The following chemicals were employed in the greenhouse studies in liquid soil treatments: Sulfuric acid, sodium dinitro-ortho-cresolate, mercuric chloride, ferrous sulfate, copper oxide, copper chloride, and aluminum sulfate. In dry soil or seed treatments the following were used: Mercuric phenyl cyanamide and cadmium oxide, mild mercurous chloride, ethyl mercury iodide, paraformaldehyde, cuprous oxide, cuprous chloride, and cuprous carbonate, ethyl mercury phosphate, finely divided metallic mercury in talc, ammonium phosphate, tetramethyl thiuramdisulfide, hydroxymercurichlorophenol, tetrachloroparabenzoquinone, sodium salt of 2-, 4-, 5-trichlorophenol, and pentachlorphenol.

Various plant materials were used also to determine whether they might either contain antibiotics against the damping-off fungi or stimulate the growth of micro-organisms antagonistic to these pathogens. These materials included ground oak leaves, pine needles, alfalfa, grass, autoclaved oats, sawdust, and corn meal. Since none were effective, further mention of them is omitted.

Nine different sets of greenhouse trials were made between 1937 and 1945 in order to determine which materials were sufficiently promising for nursery testing. Because of their volume, they are not described in detail.

From these greenhouse trials several materials appeared encourag-

ing as used, and worthy of nursery trials. The most promising were a seed dust of mercuric phenyl cyanamide and cadmium oxide and more particularly a soil application of calomel. The combination of these treatments indicated a control of damping-off approximately as good as that from sulfuric acid. However, this acid apparently killed many weeds which were not affected by these mercurials.

The possible advantages of these or similar chemicals were as follows: (1) They had no accident hazard such as that provided by pouring water into concentrated sulfuric acid. (2) They were less injurious to the skin or clothing on contact than dilute sulfuric acid. (At the same time it is clear that these chemicals are poisonous if eaten, that the dust should not be inhaled in any quantity, and that continual skin contact might be harmful.) (3) They were easier to handle. (4) They had no obviously ill effect on the fertility of the soil. Since traces of mercury had disappeared within a few months (*5*), there was no fear that the minute amount employed per square foot might accumulate.

The more promising of these substances along with certain other commonly used chemicals were employed in experimental tree nursery trials.

SMALL SCALE NURSERY TRIALS

Of the chemicals tried in the greenhouse, all were employed in small nursery beds except cuprous oxide, cuprous chloride, cuprous carbonate, and metallic mercury.

Small-bed nursery trials were made during the period 1937 to 1943, inclusive. There were two trials in nursery No. 1, seven in nursery No. 2, and four in nursery No. 3. The individually treated areas were either 2 by 2 or 3 by 4 feet in size. Each trial consisted of a number of treatments (3 to 22) and replications (4 to 6) as appeared necessary. Although most of the details of these studies have been omitted because of their volume, some of the results of a trial on inoculated soil are given in table 1, as representative of the group.

The percentages of damping-off for each of the six replicates in the three critical treatments shown in table 1 were as follows: No treatment—18, 7, 45, 11, 10, and 42; sulfuric acid—2, 8, 3, 12, 7, and 30; combination—4, 1, 3, 3, 2, and 1. No statistical analysis seemed necessary to conclude that in this trial the combination was significantly superior to no treatment and as effective as the sulfuric acid

TABLE 1.—*Averages from 6 replications of small-scale nursery trials on the control of damping-off of red pine seedlings, made in 1939, on inoculated soil at nursery No. 1* [1]

Treatment	Seed planted that emerged	Emerged seedlings that were—		
		Damped-off	Chemically injured	Healthy
	Percent	*Percent*	*Percent*	*Percent*
None	70	22	0	78
Sulfuric acid [2]	66	10	2	89
Combination [3]	83	2	0	98
Seed dust alone	80	15	0	85
Soil dust alone	84	7	0	93

[1] 11 of the less promising among 16 treatments have been omitted.
[2] 2 percent sulfuric acid, 3 gallons per 4 by 12 foot bed (235 ml. per square foot).
[3] Seed dust of mercuric phenyl cyanamide, 8 percent, and cadmium oxide, 2.5 percent (commercial Barbak C); soil dust treatment of calomel, 0.4 gm. per square foot.

against damping-off. Particularly in the sulfuric acid plots, some of the seedlings classed as damped-off may have been chemically injured.

Isolations were made from damped-off seedlings in various treatments. *Pythium* was rather less prevalent than *Rhizoctonia* (ratio 15 to 21) in the untreated plots and was a poor second to it (7 to 22) in the sulfuric acid plots. However, *Pythium* appeared exclusively (16 to 0) in the plots treated with calomel.

The combination treatment appeared sufficiently promising to justify trials on a commercial scale.

COMMERCIAL SCALE TRIALS

Commercial nursery trials were made with the best of the treatments from the 9 greenhouse and 13 small-bed nursery trials, viz, (1) sulfuric acid, (2) mercuric phenyl cyanamide with cadmium oxide seed dust, (3) calomel on the soil, and (4) a combination of (2) and (3).

The trials consisting of five treatments and six replications were made in 1940, 1941, and 1942. The field manipulations were similar to those described for the small-bed trials. The results with controls, sulfuric acid, and the seed and soil treatments alone and combined (table 2) show some of the variability encountered. The combina-

TABLE 2.—*Averages from 6 replications of large-scale nursery trials on the control of damping-off of pine seedlings*

Location, year, and pine seed	Treatment	Seed planted that emerged	Emerged seedlings that were—		
			Damped-off	Chemically injured	Healthy
Nursery No. 1:		*Percent*	*Percent*	*Percent*	*Percent*
1940 Austrian	None	27	16	0	84
	Sulfuric acid [1]	32	2	4	94
	Combination [2]	28	6	0	94
	Seed dust alone	28	7	0	93
	Soil dust alone	28	7	0	93
1941 Red	None	79	12	0	88
	Sulfuric acid	76	5	0	95
	Combination	74	1	0	99
	Seed dust alone	71	10	0	90
	Soil dust alone	77	2	0	98
1942 Scotch	None	18	30	0	70
	Sulfuric acid	16	11	2	87
	Combination	20	9	0	91
	Seed dust alone	19	25	2	73
	Soil dust alone	20	8	1	91
Nursery No. 2:					
1940 Red [3]	None	84	2	0	98
	Sulfuric acid	75	3	15	82
	Combination	80	1	0	99
1941 Red [3]	None	81	8	0	92
	Sulfuric acid	78	2	7	91
	Combination	81	2	0	98
1942 Red [3]	None	87	2	0	98
	Sulfuric acid	87	1	1	98
	Combination	86	1	0	99
Nursery No. 3:					
1940 Red	None	83	4	0	96
	Sulfuric acid	88	1	1	98
	Combination	85	1	0	99
	Seed dust alone	81	3	0	97
	Soil dust alone	85	1	0	99
1941 Red	None	76	25	0	75
	Sulfuric acid	74	13	2	85
	Combination	70	19	0	81
	Seed dust alone	71	41	1	58
	Soil dust alone	73	30	1	69

[1] 2 percent sulfuric acid, 3 gallons per 4 by 12 foot bed (235 ml. per square foot).

[2] Seed dust of mercuric phenyl cyanamide, 8 percent, and cadmium oxide, 2.5 percent (commercial Barbak C); soil dust treatment of calomel, 0.4 gm. per square foot.

[3] Results from seed dust alone and soil dust alone are omitted, because there was too little damping-off to indicate effectiveness.

tion treatment, and frequently the calomel soil dust alone, caused less chemical injury to seedlings than sulfuric acid and was as effective in controlling damping-off. On the other hand, sulfuric acid eliminated many weeds that were not affected by the other treatment.

REDUCTION IN NUMBER OF WEEDS

Because weeds often present a critical problem in the seedbed, an effort was made to find a chemical that would destroy many weed seeds, and control damping-off, without causing either injury to the pines or undesirable changes in the soil.

The following materials were tested: In liquid soil treatments, sulfuric acid and sodium dinitro-ortho-cresolate; in dry soil treatments, tetramethyl thiuramdisulfide, ethyl mercury iodide, ethyl mercury phosphate, tetra-chloro-parabenzoquinone, sodium salt of 2-, 4-, 5-trichlorophenol, mercurous chloride, and hydroxymercurichlorophenol.

These materials were employed in four trials of from three to six replications each in the greenhouse in 1941, 1942, and 1943, and in four trials each with four small-bed replications in two forest nurseries. Although several of the materials gave fair weed control, all but tetramethyl thiuramdisulfide, as used, failed to meet other requirements. A summary of portions of the field trials is given in table 3. For brevity the treatments with little promise have been omitted.

TABLE 3.—*Averages from 4 replications of soil treatments against weed seeds and damping-off in red pine seedbeds*

Soil treatment	Measure used	Amount per square foot	Weeds per 12 square feet	Seed planted that emerged	Emerged seedlings that were—		
					Damped-off	Injured by chemicals	Healthy
1942 Nursery No. 2:			*Number*	*Percent*	*Percent*	*Percent*	*Percent*
None			102	85	6	0	94
Calomel [1]	Grams	0.4	55	83	3	2	95
TMTD, pure [2]	do	.3	57	86	1	1	98
Do	do	.6	24	85	2	1	97
Do	do	1.2	9	86	1	2	97
Sulfuric acid, 2 percent	Milliliters	236	17	77	2	5	93
1942 Nursery No. 3:							
None			105	72	2	0	98
Calomel [1]	Grams	.4	85	73	2	0	98
TMTD, pure [2]	do	.3	86	64	1	1	98
Do	do	.6	24	71	1	0	99
Do	do	1.2	10	64	1	1	98
Sulfuric acid, 2 percent	Milliliters	236	5	80	0	1	99
1943 Nursery No. 2:							
None			448	80	8	0	92
Calomel [1]	Grams	.4	264	90	5	0	95
TMTD, 50 percent [2]	do	1.2	107	86	12	0	88
Do	do	2.4	58	86	7	0	93
Do	do	4.8	17	95	4	0	96
Sulfuric acid, 2 percent	Milliliters	236	63	86	[3] 25	0	75
1944 Nursery No. 2:							
None			70	76	13	0	87
TMTD, 50 percent [2]	Grams	4.8	10	61	0	0	100
Sulfuric acid, 2 percent	Milliliters	236	28	81	2	0	98

[1] Seed also treated with mercuric phenyl cyanamide and cadmium oxide (commercial Barbak C).

[2] Tetramethyl thiuramidisulfide (pure was commercial Tuads, 50 percent was commercial Thiosan).

[3] This increase in the amount of damping-off following acid treatment has never been encountered in any other trials. The readings for the 4 replications, respectively, were as follows: 23, 27, 25, and 25 percent. Similar increases appeared with 2 other chemical treatments. *Rhizoctonia* was particularly severe in 1943.

The amount of damping-off in these trials, especially in 1942, was not large enough to give much information about damping-off control in the nursery. However, in these trials tetramethyl thiuramdisulfide appeared equal to sulfuric acid in respect to (1) weed control, (2) control of damping-off, (3) chemical injury to seedlings, and (4) seedling emergence. The significance of the weed control was apparent from inspection.

One of these experiments (1943, nursery No. 2) was in progress during a season when winter injury was severe.

WINTER INJURY

The winter of 1943–44 was particularly severe on pine seedlings in several Wisconsin forest nurseries. There was no rain, sleet, or snow during December. The lack of adequate snow cover caused a large amount of frost heaving and winter drought. However, in the spring of 1944, some plots that had been treated and seeded in 1943 survived the winter better than others.

The treatments and seedings were made in April 1943 with three replications. The results (table 4) were significant by inspection, so no statistical analyses were made. The data indicate that the use of increasingly larger amounts of the chemical gave concomitantly improved protection against winterkilling. The results presented are on only 1 year's observations in one locality. Since such winters are unusual, the opportunities are limited for repeating such observations. The writers hope they will be tried in regions where such winter injury is common. At the same time the influence of hydrophilic colloids in the soil (*1*) deserves consideration.

TABLE 4.—*Summary of survival of red pine after various soil treatments in relation both to damping-off and to winter injury*

Soil treatment	Year	Measure used	Amount per square foot	Seedlings from seed planted
				Percent
None:				
Emergence, June and July	1943			88
Seedlings living, August	1943			81
Seedlings living, July	1944			33
Sulfuric acid (2 percent):				
Emergence, June and July	1943	milliliters	235	86
Seedlings living, August	1943	do	235	64
Seedlings living, July	1944	do	235	20
Calomel [1]:				
Emergence, June and July	1943	grams	4	90
Seedlings living, August	1943	do	4	85
Seedlings living, July	1944	do	4	33
Tetramethyl thiuramdisulfide:				
Emergence, June and July	1943	grams	1.2	86
Seedlings living, August	1943	do	1.2	76
Seedlings living, July	1944	do	1.2	63
Tetramethyl thiuram disulfide:				
Emergence, June and July	1943	grams	2.4	86
Seedlings living, August	1943	do	2.4	80
Seedlings living, July	1944	do	2.4	71
Tetramethyl thiuramdisulfide:				
Emergence, June and July	1943	grams	4.8	93
Seedlings living, August	1943	do	4.8	92
Seedlings living, July	1944	do	4.8	83

[1] Seed also treated before planting with mecuric phenyl cyanamide and cadmium oxide.

DISCUSSION

The variability inherent in the damping-off problem has indicated that any control measure may give fluctuating results from place to place in the same year and from year to year in the same place. In Wisconsin, the activity of the two principal fungi changed with different circumstances. For example, *Pythium* ordinarily was active in cool, wet, and slightly acid to neutral soil, while *Rhizoctonia* appeared in warm, moderately dry, and relatively acid soil (*7*, *8*, *9*). The Plainfield sand is light enough to absorb rain easily and to blow when not protected. In another locality and with a different kind of soil, one might expect these or different pathogenic fungi to operate in various ways—and correspondingly that other control measures might be necessary.

An increase in damping-off has appeared from time to time when the chemical treatment was quite mild. Examples appear in table 2 (nursery No. 3, 1941 trials), in the 1943 trials of table 3, and have been encountered frequently among chemical treatments not recorded because they were not effective against damping-off. Of the factors that might influence this increase, two deserve special mention.

Chemical injury and damping-off are difficult enough to distinguish when they are operating separately. As Roth and Riker (*7*) have pointed out, seedlings that have been weakened may be more susceptible to damping-off than vigorous seedlings. As a result, under certain conditions a chemical injury too mild to be classed as such may open the way for damping-off fungi and thus provide more damage than no treatment at all.

A more complicated consideration involves antagonisms between different biological agents in the soil. Damping-off fungi have to compete against other kinds of soil fauna and flora, including *Trichoderma*. Perhaps some of the mild chemicals injure or destroy the inhibitors of the damping-off fungi and, thus, indirectly favor the development of damping-off.

The effectiveness of tetramethyl thiuramdisulfide is difficult to explain. Possibly its well-known antioxidational properties would particularly affect the *Rhizoctonia*. Further study is suggested on its repellent or destructive effect on soil fauna. For example, if it inhibited worms that attacked roots, and flagellates that killed nitrifying bacteria, the seedlings would have improved water-absorbing systems and a better nutritive balance to withstand winter injury. If further trials were made with this chemical, the related but less expensive commercial "Thiuram M" might be included.

While the authors have achieved their purpose in finding chemicals, in addition to sulfuric acid, that control damping-off and that do not obviously upset the nutrient balance in the soil, the question of relative costs is quite pertinent. These chemicals cost more than sulfuric acid. However, they need less labor to apply, and they require no supplementary application of fertilizer to restore the desirable base exchange capacity disturbed by the acid. Counting everything, the cost of using these chemicals seems close enough to that of sulfuric acid to deserve consideration.

In any case, it seems better, when possible, to avoid damping-off by such cultural means as may best suit the circumstances; e. g., (1) the use of newly cleared land, (2) fall seeding, or (3) late spring seeding with the seed covered by sand rather than soil.

SUMMARY

A search has been made for a means of controlling damping-off in Wisconsin forest nursery seedlings which would be as effective as dilute sulfuric acid, but which would not obviously upset the nutritional balance in the soil.

A seed treatment of mercuric phenyl cyanamide with cadmium oxide, supplemented by a soil treatment with calomel, was relatively effective against damping-off from *Pythium* and *Rhizoctonia* in Wisconsin's sandy soil.

A soil treatment with tetramethyl thiuramdisulfide in limited trials was relatively effective not only against this damping-off but also against many weed seeds and winter injury.

LITERATURE CITED

(1) Felber, I. M., and Gardner, V. R.
1944. effect of a hydrophilic colloid of high viscosity on water loss from soils and plants. Mich. Agr. Expt. Sta. Tech. Bul. 189, 30 pp., illus.

(2) Hartley, C.
1921. damping-off in forest nurseries. U. S. Dept. Agr. Bul. 934, 99 pp., illus.

(3) ——— Merrill, T. C., and Rhoads, A. S.
1918. seedling diseases of conifers. Jour. Agr. Res. 15: 521–558, illus.

(4) Johnson, A. G., Leukel, R. W., and Haskell, R. J.
1944. treat seed grain. U. S. Dept. Agr. Misc. Pub. 219, 12 pp., illus.

(5) Monteith, J., Jr., and Dahl, A. S.
1932. turf diseases and their control. U. S. Golf Assoc. Green Section Bul. 12: 86–186, illus.

(6) Riker, A. J., Gruenhagen, R. H., and Roth, L. F.
1943. control of damping-off of nursery pine seedlings. (Abstract) Phytopathology 33: 11.

(7) Roth, L. F., and Riker, A. J.
1943. life history and distribution of pythium and rhizoctonia in relation to damping-off of red pine seedlings. Jour. Agr. Res. 67: 129–148, illus.

(8) ——— and Riker, A. J.
1943. influence of temperature, moisture, and soil reaction on the damping-off of red pine seedlings by pythium and rhizoctonia. Jour. Agr. Res. 67: 273–293, illus.

(9) ——— and Riker, A. J.
1943. seasonal development in the nursery of damping-off of red pine seedlings caused by pythium and rhizoctonia. Jour. Agr. Res. 67: 417–431, illus.

(10) Wilde, S. A.
1937. recent findings pertaining to the use of sulfuric acid for the control of damping-off disease. Jour. Forestry 35: 1106–1110.

SOME PHYSIOLOGICAL CHARACTERISTICS OF FOUR VARIETIES OF SPRING WHEAT PRESUMABLY DIFFERING IN DROUGHT RESISTANCE [1]

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INTRODUCTION

Varieties of wheat, *Triticum aestivum* L. (*T. vulgare* Vill.),[3] differ in drought resistance, as indicated by their yields of grain and their growth characteristics under semiarid or drought conditions. To determine to what extent the supposed differences in drought resistance of Baart, Ceres, Marquis, and Hope wheats are correlated with the moisture content of the leaf blades and with the osmotic pressure and total solids of the extracted leaf-blade juices, the growth characteristics of these four varieties were studied at Tucson, Ariz., in irrigated and nonirrigated (drought) plots, 1934 to 1938.

REVIEW OF LITERATURE

Drought resistance, according to Maximov (*10, p. 399*),[4] is the "capacity of plants to endure drought and to recover readily after permanent wilting, with the minimum of damage to the plant itself

[1] Received for publication November 8, 1944. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Arizona Agricultural Experiment Station. Adapted from a thesis submitted to the Graduate College of the University of Arizona in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] Acknowledgment is made to Dr. L. M. Pultz, head, Department of Botany and Range Ecology, Arizona Agricultural Experiment Station, for highly useful suggestions in the preparation of this paper and in the interpretation of the data recorded herein; and to Dr. R. S. Hawkins, head, Department of Agronomy, Arizona Agricultural Experiment Station, for criticisms during the period of experimentation.

[3] *Triticum aestivum*, originally applied by Linnaeus to the bearded spring wheats only, under the International Rules of Botanical Nomenclature becomes the specific name for all common bread wheats, since it was the earliest name applied to any of the varieties of this group. The concept of this species as emended by Host under the name *T. vulgare* Vill. is that now most generally accepted by wheat specialists. Wheat specialists likewise have preferred the name *T. vulgare* because it is more truly descriptive of the species. The name *T. vulgare*, however, is untenable under present rules of nomenclature, since it was originally proposed by Villars merely to replace the earlier name, *T. aestivum*. It is hoped, however, that at an appropriate time the rules can be amended so that the name *T. vulgare* can be conserved for this species.

[4] Italic numbers in parentheses refer to Literature Cited, p. 111.

and to the yield produced." Briggs and Shantz (*2*) stated that permanent wilting in plants occurs when the moisture content of the soil in which they are growing is reduced to the point where the plants will not recover when placed in a water-saturated atmosphere. They will, however, slowly recover after the addition of water to the soil. Such wilting is injurious to the plants (*10*), especially to the embryonic tissue of the stem and to the root hairs.

The rates of water loss of cut plants of Baart and Ceres wheat were found by Bayles, Taylor, and Bartel (*1*) to be less than those of Marquis and Hope. Martin (*9*) reported that the rate of water loss in sorghum leaves is less than in corn leaves. On the other hand, Newton and Martin (*14*) found that timothy and western ryegrass had about the same rate of water loss, even though the latter is the more drought-resistant.

Miller (*12*) reported that the leaf moisture of corn was higher than in the drought-resistant crops kafir and milo. No relations were found by Flerov, Brokert, and Levin (*4*) between the water content of spring wheat varieties and their drought resistance. Schopmeyer (*15*) found that the drought-resistant shortleaf pine had a higher leaf-moisture content and a lower osmotic pressure than the non-resistant loblolly pine, even when the soil moisture was at the wilting point. In the lowland prairie, where soil moisture was abundant, Marsh (*8*) found no correlation between leaf moisture and osmotic pressure in the species he studied. However, a relation occurred under the dry conditions in the upland prairie.

Several workers have indicated that a high sugar content is associated with drought resistance. Sorghums have a larger amount of reducing and nonreducing sugars than corn, according to Miller (*13*). As the intensity of drought increased, Vasiliev (*16*) found in wheat plants, first accumulation of sucrose; then hydrolysis of sucrose to monosaccharides; and finally, as complete desiccation occurred, disappearance of both sucrose and monosaccharides. Lvoff and Fichtenholz (*6*) observed that drought-resistant plants possess a greater power of hydrolyzing starch to monosaccharides than do susceptible plants.

Maximov (*10*) stated that a high osmotic pressure is generally characteristic of xerophytes and increases drought resistance by (1) protecting the protoplasm from coagulation and desiccation during wilting owing to the accumulation of salts and organic substances in the sap; (2) preventing wilting for a long time in the presence of a water deficit because of the tension on the mesophyll cells; and (3) enabling resistant plants to obtain moisture more readily from a very dry soil by creating a high suction power.

Martin (*9*) observed a higher osmotic pressure in the roots and stalks of sorghum than in those of corn, but the reverse in the case of the leaves. Newton and Martin (*14*) reported that generally a higher osmotic pressure was found in drought-resistant wheats and grasses than in those not resistant; however, they did not consider the osmotic pressure a major factor in determining drought resistance. Schopmeyer (*15*) arrived at similar conclusions. Meyer (*11*) concluded that the osmotic pressure could not be safely used in central Ohio to differ-

entiate between habitats. Korstian (*5*) found that moisture had a greater influence on the osmotic pressure of the cell sap than either light or temperature.

MATERIAL AND METHODS

The experiments reported here were confined to four varieties of spring wheat, namely Baart, Ceres, Marquis, and Hope, chosen because of their range in field behavior under drought conditions, presumably indicating differences in drought resistance. Baart is a white wheat, which generally produces a high grain yield in central Washington, in the high plateau lands of eastern Idaho, and in other far western locations where the yearly rainfall is below 15 inches. According to Clark (*3*), it is the most drought-resistant wheat grown in the United States. The other three are hard red spring varieties. Ceres has outyielded Marquis under the dry conditions of the northern Great Plains. Hope, although highly resistant to the wheat rusts, has been shown to be susceptible to drought injury under severe conditions.

Waldron (*17*) reported average yields of 16.3, 14.1, and 12.7 bushels per acre for Ceres, Marquis, and Hope, respectively, in 25 trials under generally dry, rust-free conditions in Montana and western North Dakota and South Dakota. Later (*18*) he reported Hope and its sibs to be very susceptible to hot, dry weather. Ceres was highly resistant to these conditions, and Marquis was intermediate.

These varieties were grown at the University Farm, Tucson, Ariz., under two environments established respectively in two comparable, adjacent large plots surrounded by levees, one plot irrigated and the other having no irrigation water applied after seeding and hence subjected to severe drought. These two environments are called the irrigated plot and the drought plot, respectively. The same plots were used each year. The irrigated plot was irrigated several times after seeding, as needed to keep the wheat growing in a normal condition so far as could be judged from the appearance of the wheat plants. The border method of irrigation was used throughout. The dates of these irrigations are shown later.

Since the field on which these plots were located is uniform and since the plots were adjacent to each other, it is a reasonable assumption that most of the differences in osmotic pressure, total solids, and leaf moisture are due directly or indirectly to differences in soil moisture. Some leaching of nitrates may have occurred in the irrigated plot during the growing season.

The soil on which these tests were conducted is classified as Gila loam, dark-colored phase, and has a moisture equivalent of 23.5 percent. The moisture content at the point of permanent wilting is 12.8 percent. The seedings were made on December 10, 1933, February 18, 1935, January 22, 1936, and January 26, 1938. No planting was made in 1937.

The first collections each year were made from plants in the irrigated plot. None was made in the drought plot until the plants showed a slight need for water. With the exception of 1935, the first collections from the drought plot were made soon after the plants in

the irrigated plot had received the first irrigation. In the early collections, when only one or two leaves of the wheat plants were fully extended, the samples for the laboratory determinations were gathered by cutting off the entire plant as close to the ground as possible. Later collections consisted of the top three leaves of the plants. When the second or third leaves from the top had started to dry in the drought plot, the samples from both plots consisted of only the top leaf or the top two leaves. The same sampling procedure was followed in both the irrigated and the drought plot. Final collections were made just before the top leaves in the drought plot had started to dry up. This was about 3 weeks or more before the plants reached maturity in the irrigated plot. No dead or partially dead leaves were collected at any time in either plot.

Except in 1934, the collections were started at 1:30 p. m. and generally were made every 4 days. The samples were taken to the laboratory and treated according to the method of Mallery (7) immediately after collection. A pressure of 10,000 pounds per square inch, obtained with a Carver hydraulic laboratory press, was used in expressing the cell sap from the heated leaf samples. A portion of each sample of sap was used for the determination of osmotic pressure by standard cryoscopic methods and the remainder for measuring the percentage of total solids with a refractometer.

For the leaf-moisture determinations in 1935 and in 1936, 2- to 4-gm. samples were dried to constant weight at 100° C. In 1938, 5- to 10-gm. samples were used and were dried at 80°. No determinations of leaf moisture were made in 1934.

Soil samples to a depth of 4 feet were taken for moisture determination in both the irrigated and the drought plot at three or more intervals during each of the 3 years. These samples were dried to constant weight at 100° C.

Observations made in the drought plot showed that in the last 3 years the crown roots were either lacking or were very weakly developed. Occasionally, when the leaves were pulled off the plants for cryoscopic determinations, entire plants would be pulled out of the ground. There seemed to be no varietal differences in this respect. Most of the plants had only a single culm. The plants in the drought plot were half to two-thirds the height of comparable irrigated plants at maturity. As a rule, the lower leaves of plants of the former were but slightly smaller than those of the latter. However, the top leaves in the drought plot were about half the size of those in the irrigated plot. All the varieties in the drought plot flowered each year, and some seed was produced each year except 1936. Yields, however, were very low because of severe drought injury. The plants of Baart flowered about 10 days earlier than those of Ceres, Marquis, and Hope. There was an abundance of waxy bloom on the leaves and leaf sheaths of Ceres. In the development of bloom the varieties ranked in the order Ceres, Baart, Marquis, and Hope. The wilting of the leaves was so gradual that it was difficult to observe any major changes. In the later stages, the top leaf in many Baart plants was entwined about the culm.

Bound-water determination made on samples of some of the plants gave such erratic results that the data are not reported.

WEATHER CONDITIONS

The precipitation and mean temperature for each month from January to May for each of the years in which experiments were conducted are shown in table 1. The precipitation during the 4 years was lowest in 1934 and highest in 1935. There were no large differences in the mean monthly temperatures for the different years.

TABLE 1.—*Precipitation and mean temperature for each month during 4 growing seasons at Tucson, Ariz.*

Month	Precipitation				Mean monthly temperature			
	1934	1935	1936	1938	1934	1935	1936	1938
	Inches	*Inches*	*Inches*	*Inches*	*°F.*	*°F.*	*°F.*	*°F.*
January	0.50	1.25	0.96	0.65	49.3	52.1	48.8	52.4
February	.30	2.43	.92	.88	56.4	55.0	53.2	54.2
March	.39	1.46	.55	.43	63.9	55.0	59.0	57.6
April	.03	[1] T	.07	.08	68.6	64.7	66.9	65.3
May	.05	.14	T	.11	78.4	67.6	75.3	71.3
Total or mean	1.27	5.28	2.50	2.15	63.3	58.9	60.6	60.2

[1] T=trace.

SOIL MOISTURE

The percentages of soil moisture in the surface 4 feet of soil for 1935, 1936, and 1938 in the irrigated and drought plots are shown in table 2. Soil moisture in the irrigated plot was kept above the wilting percentage (12.8 percent) except in the surface foot, where it fell slightly below in 1936 and 1938.

With few exceptions the moisture content of the soil at the first samplings each year was about the same in the two plots, but at the last sampling dates for each season the moisture content of the soil in the drought plots usually was 0 to 7 percent less than in the irrigated plot. In the drought plots the soil moisture in the surface foot usually was below the wilting percentage except at the beginning of the season, but in the second, third, and fourth foot it usually was above.

EXPERIMENTAL RESULTS

MOISTURE CONTENT OF LEAVES

The moisture content of the leaves of all varieties, grown with and without irrigation, are shown in tables 3 and 4 and in figure 1. It will be noted that the deficit of soil moisture in the drought plot resulted in a considerable decrease in the moisture content of the leaves of all varieties as compared with those in the irrigated plot.

The average yearly difference in the percentage of leaf moisture between the irrigated and the drought plots for all sampling dates ranged from 1.6 for Ceres in 1935 to 6.8 for Marquis in 1938. There appeared to be no consistent differences between varieties in this respect. In 1935, the greatest difference (3.9 percent) was for Baart and the least (1.6 percent) for Ceres; in 1936, the greatest difference (5.0 percent) was for Marquis and the least (2.6 percent) for Baart; in 1938, the greatest difference (6.8 percent) again was for Marquis

and the least (4.0 percent) for Hope. In general, the differences increased as the season advanced and the soil-moisture deficit and need for water became greater.

TABLE 2.—*Soil moisture in irrigated* [1] *and drought plots at Tucson, Ariz.*

Date of sampling	Soil moisture under indicated treatment in—											
	Surface foot			Second foot			Third foot			Fourth foot		
	Irrigated	Drought	Difference [2]	Irrigated	Drought	Difference [2]	Irrigated	Drought	Difference [2]	Irrigated	Drought	Difference
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1935												
Mar. 12	17	16	1	17	18	−1	21	20	1	24	22	2
25	14	15	−1	16	16	0	19	20	−1	22	21	1
Apr. 4	17	14	3	20	17	3	22	19	3	24	20	4
14	17	13	4	19	16	3	22	18	4	22	20	2
24	15	12	3	20	15	5	23	18	5	20	20	0
May 1	14	11	3	15	14	1	19	18	1	21	18	3
10	20	13	7	20	15	5	24	15	9	27	17	10
20	14	9	5	15	11	4	19	12	7	20	16	4
Average	16.0	12.9	3.1	17.8	15.3	2.5	21.1	17.5	3.6	22.5	19.3	3.3
1936												
Mar. 26	15	15	0	17	17	0	18	18	0	20	20	0
Apr. 10	17	12	5	18	13	5	19	13	6	23	18	5
26	11	11	0	14	11	3	13	10	3	15	11	4
May 10	------	10	------	------	11	------	------	10	------	------	11	------
Average [3]	14.3	12.7	1.7	16.3	13.7	2.7	16.7	13.7	3.0	19.3	16.3	3.0
1938												
Mar. 20	16	11	5	17	17	0	20	17	3	21	18	3
Apr. 1	13	11	2	16	15	1	16	16	0	23	18	5
11	14	10	4	16	13	3	18	15	3	22	15	7
18	14	10	4	13	12	1	18	14	4	21	17	4
25	12	10	2	17	11	6	17	13	4	24	18	6
May 5	------	9	------	------	11	------	------	12	------	------	17	------
Average [3]	13.8	10.4	3.4	15.8	13.6	2.2	17.8	15.0	2.8	22.2	17.2	5.0
3-year average	14.7	12.0	2.7	16.6	14.2	2.5	18.5	15.4	3.1	21.3	17.6	3.8

[1] Irrigated Mar. 30, Apr. 10, Apr. 25, and May 7, 1935; Mar. 26, Apr. 15, and May 9, 1936; and Mar. 23, Apr. 7, and Apr. 25, 1938. When plots were sampled on the day irrigated, the samples were taken first.
[2] Minus value indicates lower soil moisture in irrigated plot than in drought plot.
[3] Averages for comparable sampling dates only.

TABLE 3.—*Differences in moisture content of leaves in 4 varieties of wheat grown in irrigated* [1] *and drought plots at Tucson, Ariz.*

Date of sampling	Baart [2]	Ceres [2]	Marquis [2]	Hope	Date of sampling	Baart	Ceres	Marquis	Hope
1935	*Percent*	*Percent*	*Percent*	*Percent*	*1936*—Con.	*Percent*	*Percent*	*Percent*	*Percent*
Apr. 17	3	0	−2	1	Apr. 19	2	6	6	4
21	2	0	2	0	23	8	1	9	8
29	5	4	1	4	27	7	6	8	6
May 7	3	3	2	1	May 1	4	8	7	7
11	−1	−4	4	6					
15	5	2	2	1	*1938*				
19	10	6	7	6	Apr. 7	2	4	4	3
					11	5	6	6	3
1936					19	6	6	8	2
Apr. 7	−3	−1	1	2	28	5	6	7	4
11	−1	1	0	3	27	5	8	8	6
15	1	3	4	3	May 5	8	8	8	6

[1] See footnote 1, table 2.
[2] Minus value indicates a higher leaf moisture in drought plot than in irrigated plot.

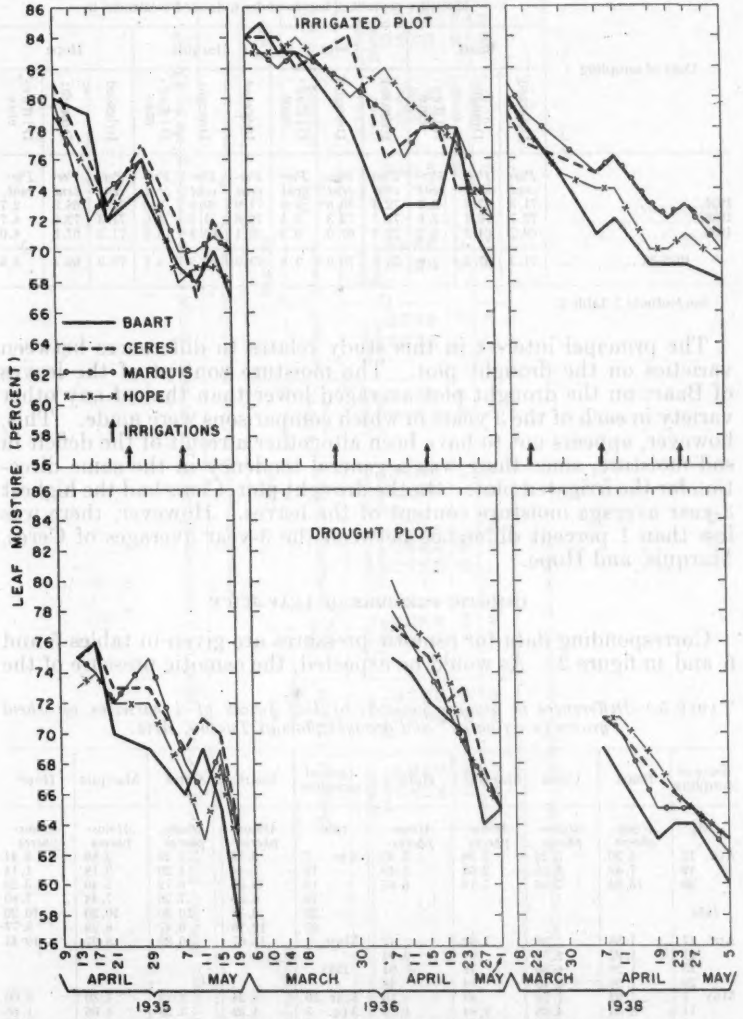

FIGURE 1.—Moisture content of leaves of four varieties of wheat grown in irrigated and drought plots at Tucson, Ariz.

TABLE 4.—*Average moisture content of leaves in 4 varieties of wheat grown in irrigated* [1] *and drought plots at Tucson, Ariz.*

Date of sampling	Moisture content of leaves under indicated treatment in—											
	Baart			Ceres			Marquis			Hope		
	Irrigated	Drought	Difference	Irrigated	Drought	Difference	Irrigated	Drought	Difference	Irrigated	Drought	Difference
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1935	71.3	67.4	3.9	72.4	70.8	1.6	71.9	69.6	2.3	71.0	68.3	2.7
1936	72.7	70.1	2.6	75.7	72.3	3.4	76.6	71.6	5.0	77.3	72.6	4.7
1938	69.7	64.5	5.2	73.3	67.0	6.3	73.1	66.3	6.8	71.3	67.3	4.0
1935–38	71.2	67.3	3.9	73.8	70.0	3.8	73.9	69.2	4.7	73.2	69.4	3.8

[1] See footnote 1, table 2.

The principal interest in this study relates to differences between varieties on the drought plot. The moisture content of the leaves of Baart on the drought plot averaged lower than that of any other variety in each of the 3 years in which comparisons were made. This, however, appears not to have been altogether a result of the deficit in soil moisture, since there was a general tendency in the same direction for the irrigated plot. On the drought plot, Ceres had the highest 3-year average moisture content of the leaves. However, there was less than 1 percent difference between the 3-year averages of Ceres, Marquis, and Hope.

OSMOTIC PRESSURE OF LEAF JUICE

Corresponding data for osmotic pressures are given in tables 5 and 6 and in figure 2. As would be expected, the osmotic pressure of the

TABLE 5.—*Differences in osmotic pressure of leaf juices of 4 varieties of wheat grown in irrigated* [1] *and drought plots at Tucson, Ariz.*

Date of sampling	Baart	Ceres	Marquis [2]	Hope [2]	Date of sampling	Baart	Ceres	Marquis	Hope
1934	*Atmospheres*	*Atmospheres*	*Atmospheres*	*Atmospheres*	*1936*	*Atmospheres*	*Atmospheres*	*Atmospheres*	*Atmospheres*
Apr. 12	5.20	5.24	2.58	3.95	Apr. 7	2.58	3.24	2.88	3.44
19	7.49	5.35	2.62	3.49	11	1.44	1.20	3.18	1.14
29	15.53	7.98	7.14	6.66	15	3.36	3.72	5.46	5.22
1935					19	6.36	7.26	7.44	7.80
					23	9.78	10.20	10.20	10.20
Apr. 17	1.56	.36	1.32	−.42	27	12.10	6.47	8.39	9.77
21	1.92	2.58	.84	−2.04	May 1	15.67	10.30	8.27	10.43
25	1.26	1.86	.72	−.84	*1938*				
29	1.56	2.46	.54	.48					
May 7	1.92	1.74	.48	−.16	Mar. 30	3.24	3.72	3.30	3.60
11	3.85	4.08	2.64	1.62	Apr. 3	4.52	3.23	3.06	1.66
15	5.70	4.32	2.58	.96	7	.15	.28	.44	1.97
19	3.96	4.80	−2.10	−.90	11	4.34	3.32	5.08	3.88
					19	6.24	4.96	5.46	5.32
					23	1.70	.84	.40	.73
					27	6.63	3.04	3.60	4.48
					May 5	9.30	3.84	3.47	3.92

[1] See footnote 1, table 2.
[2] Minus value indicates a higher osmotic pressure in irrigated plot than in drought plot.

TABLE 6.—*Average osmotic pressure of the leaf juices of 4 varieties of wheat grown in irrigated [1] and drought plots at Tucson, Ariz.*

Date of sampling	Osmotic pressure of leaf juice under indicated treatment in—											
	Baart			Ceres			Marquis			Hope		
	Irrigated	Drought	Difference	Irrigated	Drought	Difference	Irrigated	Drought	Difference	Irrigated	Drought	Difference [2]
	Atmospheres	*Atmospheres*	*Atmospheres*	*Atmospheres*	*Atmospheres*	*Atmospheres*	*Atmospheres*	*Atmospheres*	*Atmospheres*	*Atmospheres*	*Atmospheres*	*Atmospheres*
1934	17.82	27.23	9.41	15.51	21.70	6.19	16.97	21.08	4.11	16.35	21.05	4.70
1935	16.63	19.35	2.72	16.30	19.08	2.78	16.35	17.23	.88	16.92	16.76	−.16
1936	17.35	24.68	7.33	16.32	22.38	6.06	15.65	22.20	6.55	15.00	21.86	6.86
1938	20.14	24.66	4.52	19.43	22.33	2.90	19.68	22.78	3.10	19.28	22.48	3.20
1934–38	17.99	23.98	5.99	16.89	21.37	4.48	17.16	20.82	3.66	16.89	20.54	3.65
1935–38	18.04	22.90	4.86	17.35	21.26	3.91	17.23	20.74	3.51	17.07	20.37	3.30

[1] See footnote 1, table 2.
[2] Minus value indicates a higher osmotic pressure in irrigated plot than in drought plot.

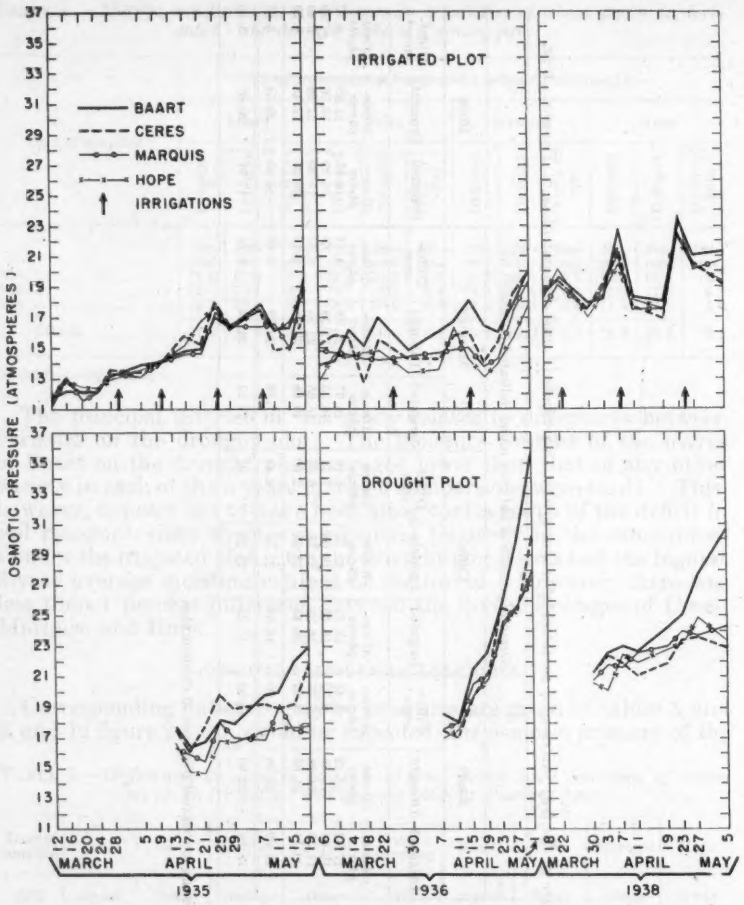

FIGURE 2.—Osmotic pressure of leaf juices of four varieties of wheat grown in irrigated and drought plots at Tucson, Ariz.

expressed leaf-blade juice was generally higher and in most cases considerably higher for the drought than for the irrigated plot. For the latter, Baart averaged higher than other varieties by about 1 atmosphere. The other three had substantially the same values. The differences, however, are not consistent from year to year, Baart averaging slightly less than Hope in 1935.

In the drought plot the osmotic pressures for Baart were rather consistently higher than those for other varieties at the later sampling dates and frequently so at the earlier dates also. As an average for all comparable sampling dates in all years, the four varieties fall into

the same order as for their assumed drought resistance, i. e., Baart, Ceres, Marquis, and Hope, the difference between Baart and Hope being 3.44 atmospheres (table 6).

TOTAL SOLIDS OF LEAF JUICE

The percentage of total solids in the expressed juice of the four varieties is shown in tables 7 and 8 and figure 3. As in the case of osmotic pressure, the total solids generally were highest for the plants in the drought plot. In the irrigated plot Baart averaged the highest and Hope the lowest for all comparable sampling dates in all years. They were also the highest and lowest, respectively, each year, though the differences in some cases were small.

TABLE 7.—*Differences in total solids of the leaf juices of 4 varieties of wheat grown in irrigated [1] and drought plots at Tucson, Ariz.*

Date of sampling	Baart	Ceres [2]	Marquis [2]	Hope [2]	Date of sampling	Baart	Ceres	Marquis	Hope
1934	*Percent*	*Percent*	*Percent*	*Percent*	*1936*	*Percent*	*Percent*	*Percent*	*Percent*
Apr. 12	2.73	2.48	1.34	1.28	Apr. 7	0.48	1.33	1.37	1.63
19	3.40	2.26	.68	.79	11	.77	1.82	.97	.98
29	6.43	3.67	3.76	3.10	15	1.39	2.23	2.76	2.36
1935					19	1.72	3.13	3.28	2.87
					23	2.87	2.25	3.99	3.81
					27	3.97	3.42	3.96	3.63
Apr. 17	.72	−.15	−.33	.14	May 1	6.08	4.03	3.89	3.39
21	.50	.24	.37	−.32	*1938*				
25	1.63	.65	.81	1.21					
29	1.08	.45	.54	1.04	Mar. 30	.77	1.47	1.55	1.34
May 7	2.36	.73	.33	.19	Apr. 3	1.75	2.03	2.22	1.69
11	2.44	1.60	1.59	.75	7	1.38	3.52	1.85	2.26
15	3.23	1.85	2.19	.86	11	2.29	2.35	3.31	2.19
19	3.02	2.13	1.54	.44	19	4.14	3.68	3.40	3.43
					23	2.97	1.89	3.05	1.71
					27	5.12	2.75	2.73	2.63
					May 5	7.63	4.54	3.62	3.20

[1] See footnote 1, table 2.
[2] Minus value indicates a higher total solids from irrigated plot than from drought plot.

TABLE 8.—*Average total solids of the leaf juices of 4 varieties of wheat grown in irrigated [1] and drought plots at Tucson, Ariz.*

Date of sampling	Total solids of leaf juice under indicated treatment in—											
	Baart			Ceres			Marquis			Hope		
	Irrigated	Drought	Difference	Irrigated	Drought	Difference	Irrigated	Drought	Difference	Irrigated	Drought	Difference
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1934	12.14	16.33	4.19	11.01	13.81	2.80	11.54	13.47	1.93	10.93	12.65	1.72
1935	10.98	12.85	1.87	10.94	11.88	.94	10.64	11.52	.88	10.40	10.94	.54
1936	9.42	11.89	2.47	9.13	11.73	2.60	8.80	11.69	2.89	8.40	11.07	2.67
1938	11.94	15.20	3.26	11.70	14.48	2.78	11.70	14.42	2.72	11.50	13.81	2.31
1934–38	11.12	14.07	2.95	10.70	12.98	2.28	10.67	12.78	2.11	10.31	12.12	1.81
1935–38	10.78	13.31	2.53	10.59	12.70	2.11	10.38	12.54	2.16	10.10	11.94	1.84

[1] See footnote 1, table 2.

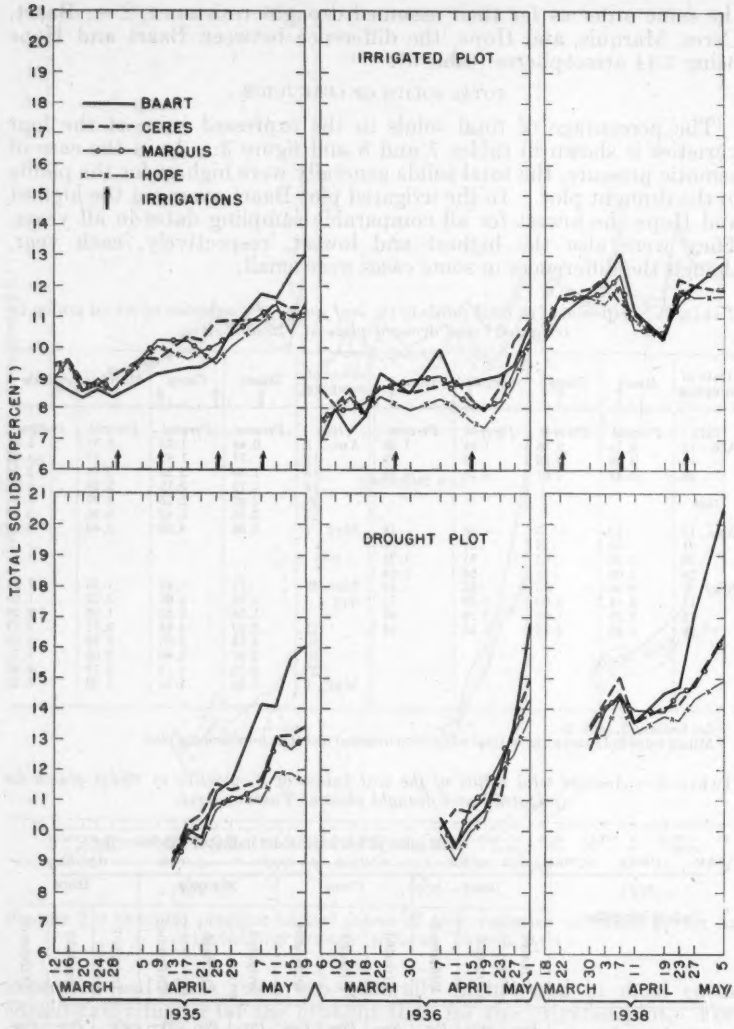

FIGURE 3.—Total solids of leaf juices of four varieties of wheat grown in irrigated and drought plots at Tucson, Ariz.

The results for the drought plot are similar except that differences are greater and more consistent. For example, Baart was always higher than Hope except for two dates, both early in the year. Like the osmotic pressures, the percentage of total solids varied in accordance with the supposed drought resistance of the varieties.

In the material from the irrigated plot, the osmotic pressure generally increased just before the irrigations were applied (fig. 2) and decreased afterward. The percentage of total solids, on the other hand, showed little if any change due to the irrigation (fig. 3). Leaf-moisture variations during these periods also were small (fig. 1). It appears probable, therefore, that the increase in osmotic pressure before the irrigations were applied was due to conversion of complex to simple carbohydrates. Likewise, some conversion of simple to complex carbohydrates seems to have occurred after the irrigations were applied.

INTERRELATIONS OF PHYSIOLOGICAL CHARACTERISTICS

Simple correlation coefficients were calculated for all data obtained each year and these are presented in table 9. Interannual coefficients for osmotic pressure and total solids for the drought plot were also calculated and these are given in table 10. In calculating these coefficients data for all varieties were pooled, since it was evident that the relations for each were substantially alike. Since soil-moisture determinations were made by plots and not according to varieties, the data for osmotic pressure, percentage of total solids, and percentage of leaf moisture of the four varieties were averaged for each date and these averages were used in calculating the correlation coefficients with soil moisture.

TABLE 9.—*Simple correlation coefficients between physiological characteristics determined on leaves of wheat plants grown in irrigated and drought plots at Tucson, Ariz.*

Treatment and items compared	1934		1935		1936		1938	
	Number	r	Number	r	Number	r	Number	r
Irrigated plot:								
Osmotic pressure and percentage of total solids	12	+0.796**	60	+0.798**	52	+0.799**	40	+0.577**
Osmotic pressure and leaf moisture			32	−.714**	48	−.681**	34	−.465**
Osmotic pressure and soil moisture			15	−.205			9	−.405
Percentage of total solids and leaf moisture			32	−.855**	48	−.754**	34	−.527**
Percentage of total solids and soil moisture			15	+.081			9	−.278
Percentage of leaf moisture and soil moisture			8	−.083			9	+.936**
Drought plot:								
Osmotic pressure and percentage of total solids	12	+.947**	36	+.832**	28	+.899**	32	+.848**
Osmotic pressure and leaf moisture			32	−.610**	28	−.885**	24	−.764**
Osmotic pressure and soil moisture			9	−.825**	7	−.888**	8	−.901**
Percentage of total solids and leaf moisture			32	−.765**	28	−.828**	24	−.703**
Percentage of total solids and soil moisture			9	−.829**	7	−.778*	8	−.861**
Percentage of leaf moisture and soil moisture			8	+.891**	7	+.954**	6	+.707

**Significant at the 1-percent level. *Significant at the 5-percent level.

TABLE 10.—*Interannual correlation coefficients for average osmotic pressure and average percentage of total solids of wheat leaf-blade juices from the drought plot*

Years compared	Osmotic pressure		Percentage of total solids	
	Number	r	Number	r
1934 and 1935	4	+0.710	4	+0.974**
1934 and 1936	4	+.992**	4	+.710
1934 and 1938	4	+.911*	4	+.988**
1935 and 1936	4	+.743	4	+.844
1935 and 1938	4	+.547	4	+.986**
1936 and 1938	4	+.982**	4	+.893*

** Significant at the 1-percent level. * Significant at the 5-percent level.

Highly significant ($P=0.01$) positive coefficients between osmotic pressure and percentage of total solids were found each year for both the irrigated and the drought plot. Of the five coefficients between soil moisture and leaf moisture, four were positive, three being highly significant. All other coefficients were negative or near zero, or both. Those between osmotic pressure and leaf moisture and between the percentages of total solids and leaf moisture were highly significant for both plots in all years.

In general, as would be expected, higher coefficients were found for the drought than for the irrigated plot, the differences in some cases being large. Thus, all coefficients for the drought plots but two were highly significant ($P=0.01$) and of the two exceptions, one was significant ($P=0.05$).

Of the six interannual coefficients (table 10) for osmotic pressure in the drought plot, two were highly significant and one was significant. Three of the coefficients for total solids were highly significant and one was significant. There appeared to be a tendency for the coefficients for total solids to be higher than those for osmotic pressure but not consistently so, that between 1934 and 1936 being distinctly less and that between 1936 and 1938 slightly less.

SUMMARY

The moisture content of the leaf blades and the osmotic pressure and percentage of total solids of the expressed juice of the leaf blades of four varieties of spring wheat, believed to differ in drought resistance and grown with and without irrigation (drought plot), were determined at several intervals in each of 3 years at Tucson, Ariz. The varieties studied were Baart, Ceres, Marquis, and Hope.

The data show that the deficit of soil moisture in the drought plot tended to decrease the moisture content of the leaves and to increase the osmotic pressure and percentage of total solids of the expressed juices.

The reduction of the moisture content of the leaves in the drought plot was about the same for all varieties, but the increase in osmotic pressure and in percentage of total solids was greatest in Baart and least in Hope. As an average for all years, the order of the varieties

with respect to osmotic pressure and percentage of total solids was the same as their supposed drought resistance: namely, Baart, Ceres, Marquis, and Hope. The average moisture content of the leaves was lowest for Baart and highest for Ceres, the differences between all varieties other than Baart being small and inconsistent from year to year.

LITERATURE CITED

(1) Bayles, B. B., Taylor, J. W., and Bartel, A. T.
1937. Rate of water loss in wheat varieties and resistance to artificial drouth. Amer. Soc. Agron. Jour. 29: 40–52, illus.

(2) Briggs, L. J., and Shantz, H. L.
1912. The wilting coefficient for different plants and its indirect determination U. S. Bur. Plant Indus. Bul. 230, 83 pp., illus.

(3) Clark, J. A.
1936. Improvement in wheat. U. S. Dept. Agr. Yearbook 1936: 207–302, illus.

(4) Flerov, K. V., Brokert, P. G., and Levin, D. I.
1930. Agrochemical characteristics of drought resistant varieties of cultivated plants. Trudy Prikl. Bot., Genet., i Selek. (Bul. Appl. Bot., Genet., and Plant Breeding) 23 (2): 111–154, illus. [In Russian. English translation.]

(5) Korstian, C. F.
1924. Density of cell sap in relation to environmental conditions in the Wasatch mountains of Utah. Jour. Agr. Res. 28: 845–907, illus.

(6) L'vov [Lvoff], S. D., and Fikhtengol'ts [Fichtenholz], S. S.
1936. [The influence of wilting on the water balance and carbohydrate metabolism in leaves of some tobacco varieties of different degrees of drought resistance.] Bot. Inst. Akad. Nauk SSSR Trudy, Éksper. Bot. (Inst. Bot. Acad. Sci. USSR. Acta, Bot. Expt.) Ser. 4 (2): 149–223. [In Russian. Abstract in Expt. Sta. Rec. 78: 23. 1938.]

(7) Mallery, T. D.
1934. Comparison of the heating and freezing methods of killing plant material for cryoscopic determinations. Plant Physiol. 9: 369–375.

(8) Marsh, F. L.
1940. Water content and osmotic pressure of certain prairie plants in relation to environment. Nebr. Univ. Studies 40, No. 3, 44 pp., illus. (Thesis, Ph. D.)

(9) Martin, J. H.
1930. The comparative drought resistance of sorghums and corn. Amer. Soc. Agron. Jour. 22: 993–1003.

(10) Maximov, N. A.
1929. The plant in relation to water. . . [Authorized English translation. Edited by R. H. Yapp. London.] 451 pp., illus.

(11) Meyer, B. S.
1927. Studies on the physical properties of leaves and leaf saps. Ohio Jour. Sci. 27: 263–288.

(12) Miller, E. C.
1917. Daily variation of water and dry matter in the leaves of corn and the sorghums. Jour. Agr. Res. 10: 11–46, illus.

(13) ———
1924. Daily variation of the carbohydrates in the leaves of corn and the sorghums. Jour. Agr. Res. 27: 785–808, illus.

(14) Newton, R., and Martin, W. M.
1930. Physico-chemical studies on the nature of drought resistance in crop plants. Canad. Jour. Res. Sect. C. Bot. Sci. 3: 336–427 illus.

(15) SCHOPMEYER, C. S.
1939. TRANSPIRATION AND PHYSICO-CHEMICAL PROPERTIES OF LEAVES AS RELATED TO DROUGHT RESISTANCE IN LOBLOLLY PINE AND SHORTLEAF PINE. Plant Physiol. 14: 447–462, illus.
(16) VASILIEV, I. M.
1931. INFLUENCE OF DROUGHT ON THE TRANSFORMATION OF CARBOHYDRATES IN WHEATS. Trudy Prikl. Bot., Genet., i Selek. (Bul. Appl. Bot., Genet., and Plant Breeding) 27: 47–69, illus. [In Russian. English summary, pp. 68–69.]
(17) WALDRON, L. R.
1931. FROST INJURY TO SPRING WHEAT WITH A CONSIDERATION OF DROUTH RESISTANCE. Amer. Soc. Agron. Jour. 23: 625–637.
(18) ———
1933. YIELD AND PROTEIN CONTENT OF HARD RED SPRING WHEAT UNDER CONDITIONS OF HIGH TEMPERATURE AND LOW MOISTURE. Jour. Agr. Res. 47: 129–147.

U. S. GOVERNMENT PRINTING OFFICE: 1947